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MOLD FLORA ASSOCIATED WITH SHELLED CORN IN IOWA[1]

R.W. Lichtwardt[2], G.L. Barron, and L.H. Tiffany

A large number of microorganisms can be found associated with deteriorating grain. In order to understand and experiment with deterioration processes, it is necessary to determine which of the organisms are important to spoilage and which are relatively insignificant or secondary. A study of deterioration in stored shelled corn, started in the fall of 1955, has led to the identification of a relatively large number of fungi associated with corn kernels in Iowa. The present paper is concerned primarily with listing and commenting on the species we have found, and indicating in a general way the frequency of occurrence of these fungi. A subsequent publication will discuss the role that some of these fungi play in deterioration of corn in storage.

MATERIALS AND METHODS

Several methods have been used to detect fungi associated with corn kernels. A distinction has been made in all instances between fungi growing from grains which have been surface-sterilized and those growing on unsterilized kernels, the former representing the internal living molds and the latter largely the external. Many internal molds will grow out of unsterilized kernels kept in a suitable environment, but some species of internal fungi are difficult or impossible to detect when the external mold flora is prolific, and their growth in some instances may be actually inhibited.

Samples of corn were surface-sterilized by dipping the kernels for a few seconds in 95 per cent ethanol, then soaking them in 10 per cent Clorox (5.25 per cent sodium hypochlorite) for 10 minutes, and finally rinsing in sterile distilled water. The treatment effectively kills all external molds but does not impair the germinability of the grain, and apparently has no effect on the internal molds of intact kernels. Where grain was to be ground, by a technique described below, surface sterilization was accomplished by the use of 0.1 per cent mercuric chloride instead of sodium hypochlorite.

Surface-sterilized and unsterilized kernels from each sample were placed in sterile moist chambers (shallow plastic boxes lined with moistened blotting paper) and other kernels from the sample were placed on

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Journal paper No. J-3375 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1300.

[2]Present address: Department of Botany, University of Kansas.

The authors express their appreciation to Dr. J. C. Gilman for his assistance and counsel during the progress of these investigations.

a nutrient medium in petri dishes. The use of both of these methods gave more satisfactory results than either method alone. For routine work we found Christensen's malt-salt agar quite satisfactory (3), which we made up as follows:

A)	dist. water	500 ml	B)	dist. water	500 ml
	malt extract	20 g		NaCl	60 g
	agar	20 g			

The two parts were autoclaved separately and the cold salt solution was added to the hot malt-agar mixture just before pouring, otherwise the agar may not gel properly.

In our early work an additional moist chamber for each sample was set up with surface-sterilized kernels that had been bisected longitudinally, but these showed substantially the same fungal infection as the uncut kernels, and this method was discontinued for routine examinations.

Internal molds in dried grain obviously are much more significant in most deterioration processes than are external molds, and for this reason the practice of examining unsterilized subsamples was discontinued after the first six months. Consequently, the majority of the results shown in Table 1 represent internal molds.

Different temperatures were used at first to incubate replicate moist chambers and petri dishes, but it was found that 20°C was generally most satisfactory. The incubation period was usually about ten days. Many of the samples were reincubated after the first reading to allow some fungi to mature.

A large number of the samples were analyzed quantitatively for internal molds based on a technique developed by Christensen and coworkers (3). Rather than use a Wiley mill or one of similar type, which are difficult to clean and to handle aseptically, we chose the Sargent Centrifugal Wet Mill No. 2 to grind surface-sterilized grain in 0.2 per cent agar. By proper adjustment of the mill a fine suspension of corn was obtained. This suspension was diluted in 0.2 per cent agar to known concentrations and plated on malt-salt agar, and from these plates quantitative counts of colonies were obtained. A special shield was constructed to protect the samples from contamination from the air or the mill during the milling process. The grinding parts of the mill were thoroughly cleaned and sterilized between each sample, thus avoiding contamination of one sample by another.

In selecting kernels to be surface-sterilized, random sub-samples were selected, but any obviously damaged (chewed or broken) kernels were discarded. However, no attempt was made to reject kernels with visible or invisible cracks in the pericarp, and for this reason it should not be assumed that any reference to internal molds implies that the molds necessarily penetrated through intact pericarps.

Our findings from samples of shelled corn obtained from storage bins are included. Over 850 samples were analyzed from approximately 300 bins. Thirty-one of the bins were located on farms and Commodity Credit Corporation binsites in Story County, Iowa. Samples were taken at intervals from these bins, usually every month. The period over which the bins were sampled varied according to the period of storage,

Table 1. Frequency of occurrence of fungi isolated from surface-sterilized and unsterilized corn.

	Grain surface-sterilized	Grain not surface-sterilized
Penicillium		
chrysogenum Thom	*	*
citrinum Thom	-	*
commune Thom	-	*
cyclopium series	***	***
expansum Link	*	*
frequentans Westling	*	*
funiculosum Thom	*	*
herquei Bainier and Sartory	*	*
multicolor Grigorieva-Manoilova and Poradielova	*	*
notatum Westling	*	*
oxalicum Curri and Thom	**	**
purpurogenum Stoll	*	*
rugulosum Thom	-	*
solitum Westling	*	*
stipitatum Thom	*	-
urticae Bainier	-	*
variabile Sopp	**	**
vermiculatum Dangeard	-	*
viridicatum series	***	***
Aspergillus		
alliaceus (Mangin) Thom and Church	-	*
candidus Link	**	*
elegans Gasperini	-	*
flavipes (Bainier) Thom and Church	*	*
flavus Link	**	**
fumigatus Fresenius	-	*
glaucus group	***	***
amstelodami (Mangin) Thom and Church		
chevalieri (Mangin) Thom and Church		
chevalieri var. intermedius Thom and Raper		
echinulatus (Delacr.) Thom and Church		
mangini (Mangin) Raper and Thom		
repens (Cdas.) DeBary		
restrictus G. Smith		
ruber (Bremer) Raper and Thom		
umbrosus Bain. and Sart.		
niger v. Tiegh.	**	**
ochraceus Wilhelm	*	*
sulphureus (Fres.) Thom and Church	*	*
sydowi (Bain. and Sart.) Thom and Church	*	-

The frequency ratings are as follows:
- not found. * infrequent. ** frequent. *** very frequent.

Table 1. - Continued -

	Grain surface-sterilized	Grain not surface-sterilized
Aspergillus (con.)		
terreus Thom	*	-
unguis (Emile-Weil and Gaudin) Thom and Raper	-	*
ustus (Bainier) Thom and Church		
variecolor (Berk. and Br.) Thom and Raper	*	-
versicolor (Vuill.) Tiraboschi	*	*
wentii Wehmer	*	*
Fungi Imperfecti		
Acremoniella sp.	-	*
Alternaria	**	**
tenuis Nees		
Alternaria sp.		
Arthrobotrys sp.	*	-
Botrytis cinerea Fr.	-	*
Candida pseudotropicalis (Cast.) Basgal	**	**
Cephalosporium sp.	*	-
Cephalothecium roseum Corda	-	*
Cladosporium sp.	*	-
Diplodia zeae (Schw.) Lev.	***	**
Epicoccum sp.	*	**
Fusarium spp.	***	-
Fusidium sp.	-	*
Geotrichum spp.	*	-
Gonatobotrys sp.	-	*
Helminthosporium sp.	-	*
Hormodendrum spp.	**	**
Monilia spp.	*	*
Myrothecium verrucaria Tode. and Fr.		
Nigrospora oryzae (B. and Br.) Petch	**	*
Oospora	*	*
O. sulphurea (Preuss) Sacc. and Volino		
Oospora sp.		
Papularia sphaerosperma (Pers.) van Hohnel	*	-
Papulospora sp.	*	*
Phoma sp.	*	-
Rhinotrichum (_Oidium_) _tenellum_ Berk. and Curt.	*	*
Scopulariopsis brevicaulis (Sacc.) Bainier	*	-
Sporotrichum spp.	*	-
Stemphylium lanuginosum Harz	*	-
Stigmella sp.	-	*
Trichoderma	*	*
T. viride Pers.		
T. koningi Oudemans		
Tritirachium roseum van Beyma	*	-

Table 1. - Continued -

	Grain surface-sterilized	Grain not surface-sterilized
Ascomycetes		
Chaetomium	**	**
bostrychodes Zopf.		
cochliodes Palliser		
doligotrichum Ames		
funicola Cooke		
globosum Kunze		
indicum Corda		
murorum Corda		
olivaceum Cooke and Ellis		
Gibberella zeae (Schw.) Petch	*	*
Eidamella sp.	*	*
Melanospora sp.	*	*
Microascus	**	*
cinereus (Emile-Weil and Gaudin) Curzi		
schumacheri (Hans.) Curzi		
intermedius Emmons and Dodge		
cirrosus Curzi		
variabilis Massee and Salmon		
Pyronema confluens Persoon	*	-
Sordaria fimicola (Rab.) Ces and De Not.	*	-
Byssochlamys nivea Westling	*	-
Thielavia sepedonium Emmons	*	-
Phycomycetes		
Absidia repens v. Tiegh.	*	*
Circinella muscae (Sorokine) Berlex and de Toni	*	*
Mucor	**	**
M. mucedo (L.) Fres.		
Mucor sp.		
Rhizopus nigricans Ehrenb.	**	**
Syncephalastrum racemosum (Cohn) Schroet.	*	-
Syncephalis reflexa v. Tiegh.	*	-
Thamnidium elegans Link	-	*
Other fungi		
Dictyostelium sp.	*	*
Tomentella granulata Bref. (conidial form)	*	-

The frequency ratings are as follows:
- not found. * infrequent. ** frequent. *** very frequent.

but a number of bins were sampled for almost two years. The remaining samples represent collections from bins in CCC binsites in 95 of Iowa's 99 counties. The majority of bins were circular steel structures, but bins of many types and capacities were sampled. The condition of the grain in these bins varied from excellent to extremely deteriorated, and the grain represented different harvest years.

RESULTS

All the bins sampled contained at least some infected kernels. The number of infected kernels ranged from a few per cent in excellent grain to 100 per cent in badly deteriorated samples. The outward appearance of the grain did not necessarily give an indication of the degree of internal infection, especially in the better-looking corn; some samples of "sound" corn had over 50 per cent infection by certain fungi, such as Diplodia and Fusarium. Likewise, the total number of infected kernels frequently was not correlated with the degree of deterioration, as measured by other methods. Infected kernels often contained a single species of fungus, but in grain that had undergone some spoilage it was not uncommon to find kernels infected with four or five different molds.

The molds that grew on the surface of unsterilized kernels consisted predominantly of the same species which might also be found internally, but in addition we found a number of miscellaneous fungi that appeared now and again during our examination of samples, and still other species that were isolated only once or twice. Many molds undoubtedly are present on corn kernels in storage, but their spores do not germinate or their mycelium does not grow favorably under certain conditions of grain storage and in competition with the other more commonly observed species.

A list of the internal and external fungi we have encountered is shown in Table 1. The relative frequencies of their occurrence in the corn samples examined is represented as follows:

- not found	** frequent
* infrequent	*** very frequent

This scale of relative frequencies is shown simply to indicate approximately the number of times the various genera or species were identified from the wide variety of corn samples studied. A rating of **, for instance, might mean that either the fungus was fairly abundant in some samples, or it consistently appeared in a few per cent of the grains in most samples, especially those showing at least a small degree of deterioration. The frequencies shown do not necessarily indicate the capacity of the respective fungi to deteriorate corn. Frequencies of individual species are given only for Aspergillus and Penicillium, two genera which are very important to spoilage processes.

A few more species of Aspergillus were found than of Penicillium, due in part to the nine species of Aspergillus belonging to the A. glaucus group. Within the A. glaucus group the most common species probably

were A. ruber, A. amstelodami, and A. repens, but with special isolation techniques A. restrictus was isolated from many of the samples. Difficulties were often encountered in identifying strains because of the tendency of a number of the isolates to fall morphologically between given species. In addition, strains representing morphological variants of single species were encountered, notably different isolates of A. ruber with different sizes and colors of conidial heads.

Among the major Penicillium isolates were the P. cyclopium and P. viridicatum series. We have purposely avoided designating species in these series because of the intergrading characteristics of the common isolates we have obtained from corn that do not conform satisfactorily to described species in Raper and Thom's manual. The distinction between the cyclopium and viridicatum series is primarily one of color, the former being the blue-green and the latter yellow-green. Upper standard cultural conditions we have been able to detect this color difference between our isolates without much difficulty, but on corn kernels, especially in the presence of various other fungi, the color distinction may be much more subtle. It is interesting that in deteriorated corn where species of Penicillium are abundant it is very common to find strains belonging to both the cyclopium and viridicatum series growing from the same kernel and with about the same growth rate.

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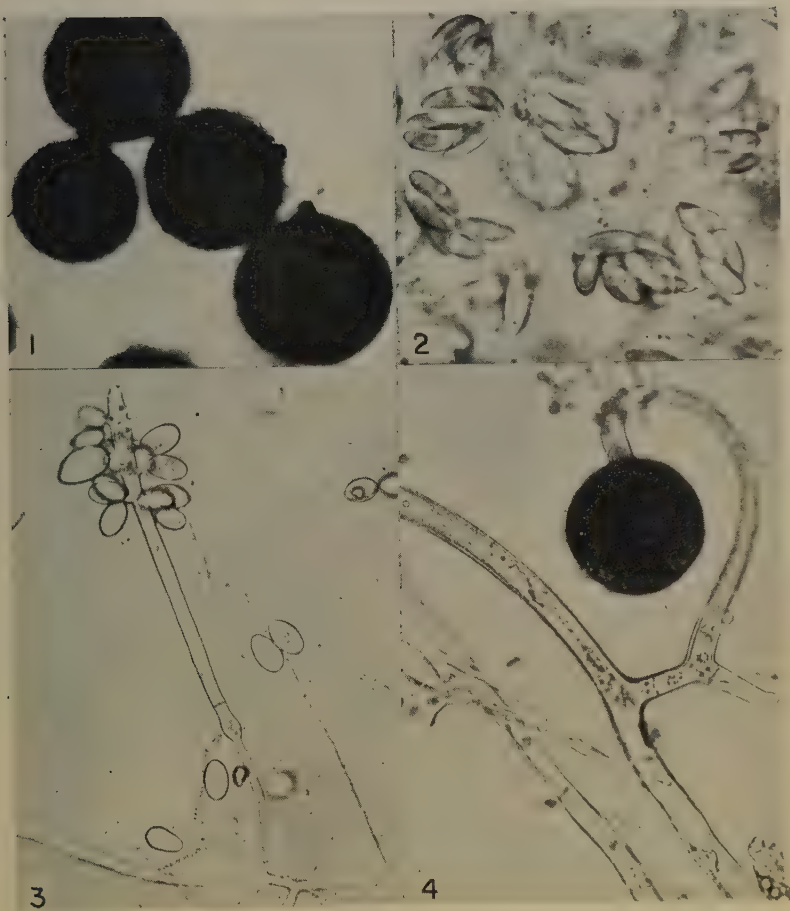
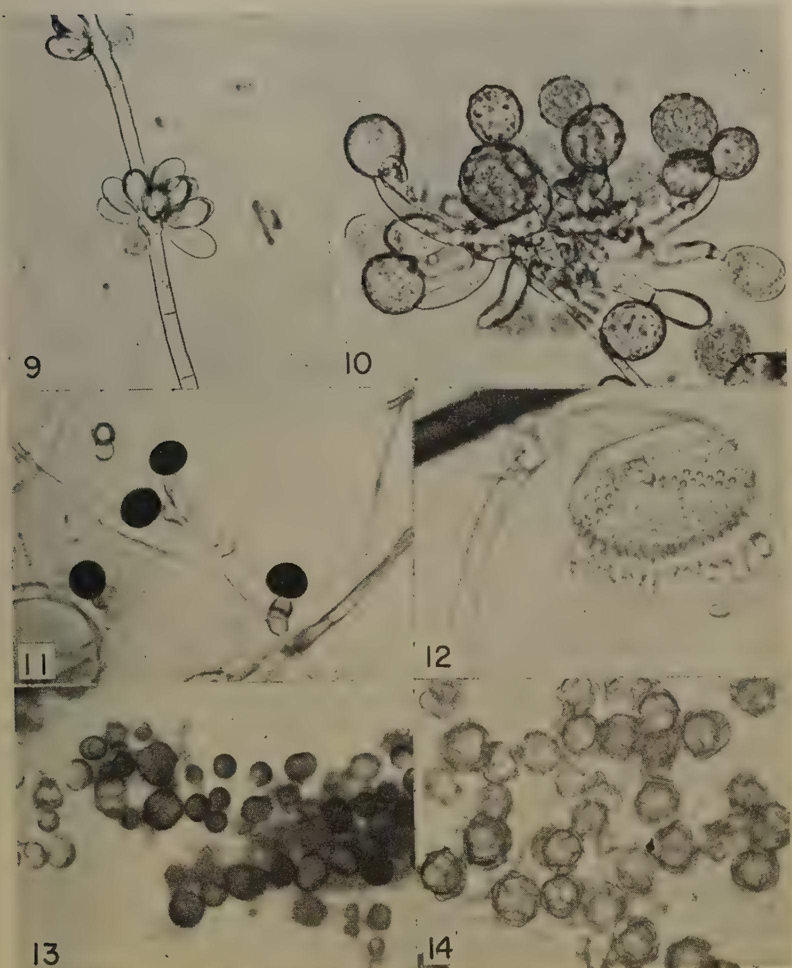


1. Perithecium, Microascus schumacheri
2. Asci and ascospores, M. schumacheri
3. Conidiophore and conidia, Rhinotrichum tenellum
4. Sporangium, Circinella muscae

5. Perithecium and portion of the cirrhus, Microascus variabilis
6. Conidiophore and conidia, Tritirachium roseum
7. Scopulariopsis conidial stage of M. variabilis
8. Perithecium and ascospores, Thielavia sepedonium



9. Conidiophore and conidia, *Gonatobotrys* sp.
10. Conidiophores and conidia, *Acremoniella verrucosa*
11. Conidiophores and conidia, *Nigrospora oryzae*
12. Sporangial head and sporangiospores, *Syncephalis reflexa*
13. Portion of the sporodochium, *Epicoccum* sp.
14. Chlamydospores, *Papulospora* sp.

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A METHOD FOR THE DETERMINATION OF PROGESTERONE IN TISSUES AND BODY FLUIDS[1]

J.M. Rakes, W.R. Hearn, and R.M. Melampy[2]

Departments of Animal Husbandry and of Chemistry
Iowa Agricultural and Home Economics Experiment Station
Iowa State College, Ames

The quantitative determination of progesterone in tissues and fluids has been accomplished by bio-assay as well as by chemical estimation. McPhail (1934) developed a quantitative procedure using the immature rabbit and Hooker and Forbes (1947) described a sensitive assay employing the response of the endometrium of the ovariectomized mouse. Zarrow and Neher (1953) have critically evaluated the Hooker-Forbes assay and have found that it will detect a concentration of 0.3 μg of progesterone per ml, which confirms the observation of Hooker and Forbes (1947). The Hooker-Forbes assay can be used to detect the small quantities of progesterone which occur in phipheral blood. However, this test is not specific for progesterone according to Zander et al. (1957).

There are several chemical procedures for the estimation of progesterone and among these are those developed by Butt et al. (1951) Bush (1952), Savard (1953), Edgar (1953), Pearlman (1954), Gawienowski (1956), Loy et al. (1957), Short (1957, 1958), and Edgar and Ronaldson (1958).

The method presented here consists of extraction and partition in different solvents, paper partition chromatography, and quantitative estimation by ultraviolet absorption spectroscopy. The identity of progesterone was confirmed in the material eluted from the chromatograms of the various tissue and fluid extracts by various chemical and physical tests. Reproductive organs, allantoic and amniotic fluids, and blood from pregnant cows were analyzed in this study. The stage of pregnancy was evaluated by the crown-rump length of the fetus according to Stoss (1944).

EXPERIMENTAL PROCEDURE

Homogenization and Extraction of Samples:

Tissues were homogenized in 95 per cent ethyl alcohol using a Lourdes homogenizer.[3] The homogenates and reproductive fluids were extracted by boiling for 1 hour with 10 volumes of 1:3 v/v diethyl ether: ethyl alcohol. Small samples of tissue were extracted in a Goldfish extractor.

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[2]Acknowledgment is made of assistance by Dr. D. T. Mayer, University of Missouri and Dr. A. M. Gawienowski, Kansas State College. Materials used were made available by Mr. J. R. Zeis, Manager, Iowa Packing Co., Des Moines, Iowa.

[3]Obtained from Lourdes Instrument Corp., Brooklyn, New York.

The extracts were filtered and concentrated over a steam bath to 50 ml and finally to 20 ml with a Rinco vacuum rotary evaporator at a temperature of 50°C.

Partition of Extracts between Organic Solvents:

The concentrated extracts were transferred to 250 ml separatory funnels. Forty ml portions of distilled water were added to the 20 ml concentrates, and insoluble material was removed. These aqueous solutions were extracted 6 times with 50 ml portions of ethyl acetate. Most of the emulsions broke on standing overnight, but in a few cases centrifugation was necessary. The combined ethyl acetate extracts were concentrated at 50°C *in vacuo*. The residues were transferred to 10 ml centrifugal tubes with 3 washings of 3 ml portions of 70 per cent aqueous methanol, and the tubes placed in a deep freeze at -12 to -15°C for overnight storage to allow cholesterol and other lipids to precipitate. Cholesterol and residual lipids were removed by centrifugation at 40,000 rpm at -15°C for 15 minutes in a Spinco model L refrigerated centrifuge using sealed 10 ml Teflon tubes[1] in a sealed type 40 rotor.

The supernates were decanted into 250 ml separatory funnels, diluted with 20 ml of distilled water, and extracted 3 times with 30 ml portions of redistilled Skellysolve B (b.p. 60-70°C). The combined extracts in each case were washed twice with 50 ml portions of distilled water. The washed Skellysolve B extracts were evaporated to dryness *in vacuo*. The evaporating flasks were rinsed 3 times with 3 ml portions of Skellysolve B, and the washings transferred to a watch glass. The Skellysolve B was removed *in vacuo* in a desiccator containing calcium chloride.

Chromatographic Separation:

The solvent system employed (petroleum ether—80 per cent aqueous methanol) was that described by Bush (1952) for the paper chromatography of steroids. Descending chromatography in a square pyrex jar[2] 12 x 12 x 24" was used. Large sheets of filter paper were placed along the inside wall of the jar to help maintain a saturated atmosphere of the mobile phase (petroleum ether, b.p. 80-100°C). A sheet of filter paper was also suspended from a solvent trough.

Preparation of Filter Paper for Chromatography:

Whatman filter paper (no.1) was cut in 17 x 60 cm sheets. These sheets were rolled, placed in a Soxhlet extractor, and extracted with absolute methyl alcohol for 72 hours to remove impurities, Butt *et al.* (1951). After drying at room temperature, each sheet was cut lengthwise into 1 cm strips spaced 1 cm apart and attached to each other at one end.

Transfer of Extracts to Chromatographic Paper:

The chromatographic strips were placed on a clean sheet of filter paper for application of the samples. A line was drawn across the strips

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[1]Obtained from Spinco Division, Beckman Instrument Co., Belmont, California.

[2]Obtained from Research Specialties and Co., Berkeley, California.

6 cm below their point of attachment and samples were spotted on this line. Leaving the strips attached facilitated placing them evenly in the trough. The strips were supported by glass rods of a nonsiphon type, which touched the paper 4 cm above the origin.

The residues on the watch glasses were dissolved in 0.2 ml of benzene, and the benzene solutions of progesterone transferred to the chromatographic strips after the benzene solution had been concentrated to a volume of 20 μl. Absolute methanol and ethanol have also been used as transfer media. The 20 μl samples were spotted on the strips in 5 μl portions using a micro-pipet and a screw-type suction pipet filler. The area covered was about 1 cm in diameter. The papers were thoroughly dried after spotting because of the strong absorbancy of benzene at 240 mμ. A blank strip was run in order to subtract the absorbancy due to solvent contamination. Progesterone standards were run on alternate strips to help locate the spots of progesterone in the tissue extracts.

Chromatographic Procedure:

The spotted strips of chromatographic paper prepared for descending chromatography were placed in a jar that had been prepared as previously described. The glass rod holder was placed on the paper strips. The lid was placed on the jar with a silicone grease seal. The jar was placed in an incubator where the temperature was maintained at 34°C. The mobile solvent was added through a hole in the lid at the end of the 24-hour equilibration period. Three hours later the sheets were removed and dried.

Location of Progesterone on Chromatograms:

The most sensitive procedure for locating small amounts of alpha, beta-unsaturated ketones is that of scanning the paper strip with ultraviolet light which delineates these compounds as dark areas against the purple fluorescent background of the paper. A scanner apparatus was constructed for this purpose utilizing a 12-inch desk lamp[1] with a fluorescent germicidal type A filament. Light of wave lengths other than the near ultraviolet was filtered out by use of a Corning no. 9683 filter.[2] The lamp was covered with cardboard except where the filter was located. Strips were passed over the filter and the spots marked with a pencil. When the solvent front was straight, as indicated by the RF values of progesterone, it was possible to draw a straight line across the strips to aid in identification. The strips were cut at 45° angles below the progesterone spot and at right angles above the spots. These small strips were placed in an eluting apparatus.

Elution:

The elution apparatus (Fig. 1) used to elute the progesterone from the chromatogram was designed after that described by Zander and Simmer (1954). A piece of 0.25 mm bore pyrex capillary tubing was sealed in one end of a 55/50 pyrex joint. A no. 2 pyrex stopcock and a

[1]Obtained from Arthur H. Thomas and Company, Philadelphia, Pennsylvania.

[2]Obtained from Corning Glass Company, Corning, New York.

Fig. 1. Apparatus used to elute progesterone from chromatogram

10 ml reservoir were sealed on to the capillary tube. The lower end of the capillary tube was bent at a 90° angle. The end was ground flat in a vertical position at a point 8 cm below the top of the joint. A rectangular glass bar 0.5 x 1 cm was suspended from a hook on the capillary to hold the paper in place for elution. The entire elution apparatus was 40 cm high with the paper suspended slightly higher than a 5 ml glass-stoppered volumetric flask. The bottom cylinder of the apparatus was placed on a 17.5 cm square plate glass.

The paper strips were placed between the capillary face and the glass bar for elution. A 5 ml volumetric flask was placed directly under the paper strip. Five ml of eluting fluid (1:3 v/v diethyl ether:absolute ethanol) was placed in the storage chamber. The stopcock was regulated to deliver 1 drop each 11 seconds. This gave an elution time of 25 to 30 minutes. Liquid was allowed to run through the capillary until exactly 5 ml of solution was eluted. The flasks were stoppered and stored in the refrigerator until read in the Beckman DU spectrophotometer.

Matched quartz cells (1 cm) were used for all determinations. The elution solvent was used in standardizing the instrument. Readings of absorbancy were obtained to establish the curve for each sample. Furthermore, readings were also taken on solutions used in eluting paper blanks in order to subtract contamination due to solvents. The maximum absorbancy was corrected for nonspecific chromagen contamination using the formula of Allen according to Loofbourow (1943).

Progesterone solutions of concentrations of 4-80 mcg/5 ml were read at 240 mμ to establish a standard curve. Progesterone solutions obeyed the Lambert-Beer law with the absorbancy being directly proportional to the concentration (Fig. 3).

Qualitative Analysis:

A modification of the Zimmerman reaction (Savard, 1953) was used as a qualitative test for determining the presence of the Δ^4, 3-keto group. A purple color indicates a positive reaction. Pooled extracts were spotted on a strip of filter paper. This strip was dipped in 2.5 normal potassium hydroxide in ethanol (freshly prepared), blotted, dipped in 2 per cent m-dinitrobenzene in ethanol, and blotted to remove excess. The strip was then developed by gently warming at 65°C in an oven.

Infrared Analysis of Purified Extracts:

Three methods were used during this investigation to obtain an infrared analysis on purified extracts of luteal, residual ovarian, placental, and adrenal tissues as well as allantoic and amniotic fluids and blood. Region I (1800-1500 cm^{-1}) is useful in identifying carbonyl functions and unsaturated centers from the positions of the band maxima (Dobriner, Katzenellenbogen, and Jones, 1953). The main fingerprinting regions are at 1670 cm^{-1} (alpha, beta-unsaturation) and at 1707 cm^{-1} (carbon-20 carbonyl). In the first method, samples were dissolved in carbon tetrachloride and evaporated on the salt plates for analysis. This method eliminated the influence of solvent and the results obtained indicated that the material was a Δ^4, 3-keto, carbon-20 carbonyl compound. In the second method of analysis, the microcell was used in which a column of carbon tetrachloride solution was aligned in the light beam. Technical

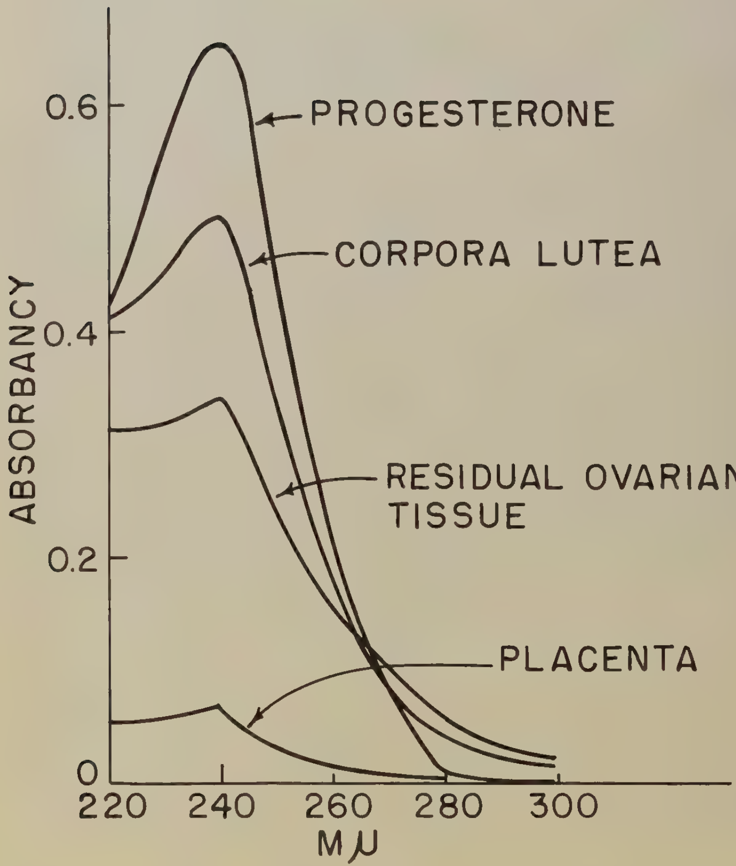

FIG. 2. ABSORBANCE OF PROGESTERONE AND TISSUE EXTRACTS

difficulties were encountered with this procedure such as maintaining a vertical column of liquid.

In the third method, the isolated progesterone was pooled in order to obtain a sufficient quantity of the hormone for analysis. The residues from pooled extracts of corpora lutea, residual ovarian tissue, placentae, adrenals, allantoic and amniotic fluids, and blood were dissolved in mineral oil and brushed on the salt plate for analysis. The oil painting method was the most successful in holding the material on the salt plates.

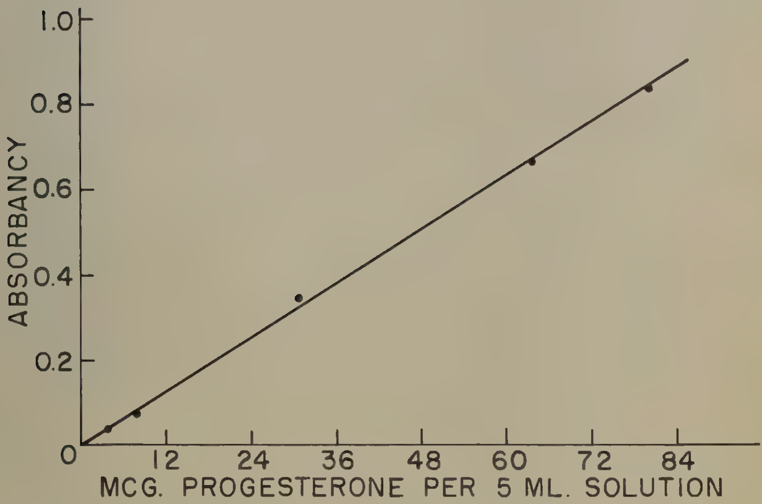

FIG. 3. ABSORBANCE OF PROGESTERONE AT 240 Mμ

Pooled extracts from 12 liters of whole blood were further purified by subjecting them to the countercurrent distribution technique used by Pearlman (1954). Eight transfers were used with a solvent system of Skellysolve B (b.p. 60-70°C); 70 per cent methanol, 3:1 v/v. Upper layers from the third, fourth, fifth, and sixth separatory funnels were pooled and dried for infrared analysis. Most of the contaminating pigments remained in the upper layers of the first, second, seventh, and eighth separatory funnels. Residues from purified extracts were dissolved in 2 drops of carbon tetrachloride and placed in the microcell for analysis. The spectra obtained indicated the presence of carbon-20 carbonyl and Δ^4, 3-keto groups characteristic of progesterone.

RESULTS AND DISCUSSION

Absorbance readings are presented in Fig. 2 for varying concentrations of pure progesterone and tissue and fluid extracts in 1:3 (v/v) diethyl ether : 95 per cent ethyl alcohol. Maxima near 240 mμ were exhibited by extracts of all tissues and fluids of the cow which were investigated. The standard curve (Fig. 3) was established using concentrations varying from 4 to 80 mcg/5 ml of solvent. Absorption spectra of progesterone and tissue extracts in 97 per cent sulfuric acid exhibited maxima near 300 mμ which suggest the presence of an alpha, beta-unsaturation according to Bernstein and Lenhard (1954). A 93 per cent recovery of progesterone was obtained with the procedure described

Table 1. Concentration of progesterone in organs and fluids of pregnant cows

Stage	Duration of pregnancy in days	No. of animals	Micrograms progesterone per gram					
			Corpus luteum*	Residual ovarian tissue	Placenta	Allantoic fluid	Amniotic fluid	Left adrenal gland
1	10-49	9	2.3 ± 0.7	1.1 ± 0.5	0.08 ± 0.04	0.06 ± 0.01	0.02*	
2	50-89	3	3.6 ± 1.5	1.0 ± 0.4	0.08 ± 0.03	0.08 ± 0.01	0.01 ± 0.003	1.5*
3	90-129	13	5.0 ± 1.2	2.7 ± 0.6	0.16 ± 0.09	0.07 ± 0.01	0.04 ± 0.01	1.7 ± 0.04
4	130-169	14	3.8 ± 0.9	1.6 ± 0.5	0.20 ± 0.06	0.07 ± 0.01	0.04 ± 0.01	1.5 ± 0.09
5	170-209	9	3.9 ± 0.7	1.9 ± 0.5	0.20 ± 0.07	0.06 ± 0.01	0.01 ± 0.002	2.1 ± 0.09
6	210-249	9	1.5 ± 0.5	0.7 ± 0.1	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.4**
7	250-280	2	1.1 ± 0.3	0.8 ± 0.5	0.03 ± 0.01	0.04 ± 0.01	0.02*	0.7**

* Mean and standard error.
** One sample

here when applied to solutions of pure progesterone. Furthermore, recovery of progesterone added to luteal tissue ranged from 75 to 80 per cent. Edgar (1953) and Short (1957) have reported recovery values of 75 and 50 per cent, respectively.

The Zimmerman reaction was positive on pooled extracts from bovine tissues and fluids. This test also indicates the presence of a Δ^4, 3-keto group characteristic of progesterone. Infrared absorption spectra of these pooled extracts from tissues and fluids indicated the presence of a carbon-20 carbonyl group as shown by a peak at 1707 cm^{-1} and a Δ^4, 3-keto group as shown by a peak at 1670 cm^{-1}.

Data obtained by using the procedure described here on various tissues and fluids from cows in different stages of pregnancy are presented in Table 1. The data in Table 1 indicate that the concentration of progesterone in the corpus luteum and residual ovarian tissue increases during the early part of pregnancy in the cow and decreases during the later stages. The hormone present in the residual ovarian tissue following removal of the corpus luteum may be due to scattered luteal cells or diffusion of the compound into surrounding tissue. The concentration of progesterone in the placenta is the greatest from the third through the sixth month of pregnancy according to the data in Table 1. Hormone concentration was greater in the allantoic than in the amniotic fluid. Progesterone concentration was the highest in the adrenal gland at about the sixth month of pregnancy. The functional significance of adrenal progesterone is unknown.

SUMMARY

A chemical method for the quantitative determination of progesterone has been developed. This procedure utilizes extraction and partition between organic solvents, paper partition chromatography, and ultraviolet absorption spectrophotometry. The identity of progesterone in the chromatograms of extracts of tissues and fluids from pregnant cows was confirmed by RF values, absorption spectra in 1:3 v/v diethyl ether, and absolute ethanol, absorption spectra in sulfuric acid, the Zimmerman reaction and infrared analysis. Data are presented and discussed on the progesterone concentration in various tissues and fluids during pregnancy in the cow.

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LEGUMINOSAE: PSORALEAE OF THE UNITED STATES A GENERIC SUMMARY[1]

Duane Isely[2]

Botany and Plant Pathology Department
Iowa State College

Perennial herbs and shrubs. Plants glandular-dotted, sometimes conspicuously so. Leaves odd-pinnate, or less frequently palmately foliolate, rarely simple. Stipules present, small, usually narrowly lanceolate. Stipels present or absent, filiform and evanescent or represented by glandular projections. Inflorescence a raceme or spike, usually densely-flowered, terminal on main stem or branches, infrequently axillary. Bracts various, usually early deciduous, subtending solitary flowers, except in Psoralea. Bracteoles present in a few groups. Calyx slightly irregular, shallowly toothed to deeply lobed, its lower calyx tooth usually the longest. Corolla various, apparently lacking in Parryella and Petalostemon, its place taken by structures of staminal origin in the latter; wings and keel partially fused to stamen tube in Dalea. Stamens 9—10 (functional stamens only 5 in Petalostemon), diadelphous or monadelphous. Fruit 1—(2) seeded, small, included or exserted from calyx, usually asymmetric, membranous to bony, indehiscent or (Psoralea in part) tending to rupture at maturity.

Some authors do not recognize the Psoraleae as a tribe but treat its members as yet another disarticulated component of the Galegeae. Rydberg (1919-20, 1928) has advocated tribal status for the Psoraleae, a viewpoint with which the writer concurs. The group can be specifically defined in terms of the glandular condition of its members and the usually 1-seeded, indehiscent or secondarily dehiscent pod. These and associated characters are not invariably present in every species, nor are such characters in any natural group. The unity of Psoraleae is further suggested by trends from one genus to another within the total complex. Such trends are often, it is suspected, properly given considerable weight by taxonomists in assaying phylogenetic units, but are not readily adaptable to diagnosis.

The Galegeae, from which the Psoraleae are removed, have historically served as a repository for those elements of the Papilionioideae

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[2]Figures by Barbara Martin Stewart.

not easily associated with presently recognized tribes. The reassignment of some of the diverse elements placed within the Galegeae is a necessary preliminary to its understanding.

The Psoraleae are primarily an American group, only Psoralea having Old World representatives. Their greatest diversity is in the southwestern United States and Mexico where not only the greatest number of species, but representatives of nearly all major phyletic units are to be found. A moderate number of species extend throughout most of the United States. Many are to be found in Central America and extend in diminishing abundance primarily along the cordillera, south to Chile. Psoralea occurs in abundance in Africa (40—50 species) and to some extent in Australia.

Three groups within the Psoraleae may be hypothesized. The first is Psoralea with unmodified papilionaceous flowers clustered 2—3 to each bract, and 10 stamens which are usually diadelphous. There is a tendency for irregular or transverse rupture of the pods in some species. The foliage may be either pinnately or palmately compound. Psoralea includes shrubs and herbs of both the New and Old Worlds. The other groups, by contrast, exhibit modifications of the corolla and androecium; the flowers are but one to each bract, fruits strictly indehiscent, and leaves odd-pinnate; they are limited to the American hemisphere.

Eysenhardtia, Amorpha, and Parryella, all woody plants, apparently constitute a series in corolla reduction, the latter completely lacking petals. The androecial sheath is likewise reduced; in the last-named genus the stamens are fused only at the very base.

Dalea and Petalostemon are associated on a number of bases, so closely that Shinners (1949) has recently merged the two genera. In Dalea, the wings and keel, in many species, arise not from the hypanthium but laterally from the stamen tube. In Petalostemon, four of the "petals" arise from the apex of the tube, this confluence of parts apparently carried one step further. However, Moore (1936), on the basis of anatomical evidence has indicated that there is no true corolla in Petalostemon, that the petaloid units are of staminal origin. Similar studies have not been conducted in Dalea, and comparative interpretation of the two genera is, therefore, presently somewhat difficult.

Chromosome numbers for a few species of the major genera have been reported (Senn, 1938; Atchison, 1949; Ledingham, 1957; Turner, 1956). On the basis of the species examined, Dalea and Petalostemon have a base number of 7, Amorpha 10, Psoralea 10 and 11. This limited data would seem to verify the presumed relationship of Petalostemon and Dalea, and point up the possible diversity of Psoralea.

Key to Genera

1. Plants woody.

   2. Petals all absent; flowers appearing yellowish. . . . . Parryella

   2. Petals (at least one) present; flowers not yellowish.

3. Only purple standard present; occurring in much of the United States. Amorpha

3. All five petals present, various in color; not found in eastern states.

4. Flowers white; scarcely papilionaceous; pods straight or falcate, well exceeding calyx. . . . . Eysenhardtia

4. Flowers usually not white, papilionaceous; pods mostly scarcely exceeding calyx. . . . . Dalea (in part)

1. Plants herbaceous.

5. Petals free from androecium; pod usually exserted from calyx; leaves palmately compound or pinnately trifoliate. . . . . Psoralea

5. Claws of wings and keel (or petaloid structures replacing them) adnate to androecium; pod included in calyx or slightly exserted; leaves odd-pinnate.

6. Functional stamens 9—10; wings and keel arising laterally from stamen tube. . . . . Dalea (in part)

6. Functional stamens 5; petaloid structures arising at apex of stamen tube. . . . . Petalostemon

Parryella T. and G. in Gray, Proc. Amer. Acad. 7:397. 1868.
Low, much branched desert shrubs. Leaves odd-pinnate, in the more common species with narrowly filiform leaflets. Stipules small or inconspicuous. Gland-based stipels are usually discernible. Flowers racemed with distinct pedicels. Bracts very small. Calyx campanulate, low-toothed. Stamens 9—10, fused below, the filaments giving a yellowish appearance to the flowers. Petals absent. Style hairy, protruding from calyx before other flower parts appear. Pod well exserted from persistent calyx, usually asymmetric, strongly glandular, one-seeded.

Two species, one of very limited distribution in Arizona, the other in Arizona, New Mexico, and southeastern Utah.

The stamens have been stated to be free from one another in this genus—this is suggested by their appearance as they extend beyond the calyx; they are, however, joined by a short sheath within the calyx in a manner akin to that of Amorpha and Eysenhardtia. The stems, leaves, and fruits are abundantly glandular, many of the glands having a hemispherical shape with a trichome-like projection.

The principal technical differences between Parryella and Amorpha are the loss of the standard and the proportionately greater length of the

free portions of the filaments in the former. The pod of Parryella is certainly suggestive of Amorpha. Possibly it represents a relatively localized, xerophytic derivative of ancestors similar to the latter, or perhaps is derived from Eysenhardtia-like plants through loss of the petals.

Amorpha L. Sp. Pl. 713. 1753. False indigo, Lead plant.

Sprawling or tall shrubs. Leaves odd-pinnate with 11—45 ovate, elliptic or shortly oblong, stalked leaflets. Stipules inconspicuous, early deciduous. Stipels usually present, narrowly lanceolate, evanescent; swollen glandular structures at base of leaflets also frequently evident. Flowers in terminal, sometimes branched, spike-like racemes; axillary racemes present in some species. Pedicels crowded, in bud subtended by early-caducous, filiform or lanceolate bracts. Calyx teeth usually short, in some species equalling tube. Keel and wings absent. Standard blue to purple, obovate or cuneate, gradually narrowing to base, somewhat rolled around filaments. Stamens 10, fused only at base, exceeding the standard. Style bearded in species examined. Pod indehiscent, often strongly glandular, obliquely beaked, curved towards standard side of flower, 0.5—2 times exceeding calyx, 1(2)-seeded.

Amorpha is a genus of perhaps 20 species whose natural distribution is limited almost exclusively to the United States. The majority of kinds occur in the southern states (Florida to California), but total distribution includes essentially all of the United States east of the Rockies and, in central North America, adjacent Canada. The most widespread species is A. fruticosa L. which is found throughout the Mississippi Valley west to Minnesota and Nebraska. It is cultivated as an ornamental somewhat beyond these limits and has been introduced in cultivation elsewhere in the world. A. canescens Pursh of the central United States was once an abundant prairie plant, and is still persistent along roadsides and in prairie remnants in the midwestern states.

Amorpha is distinctly characterized by its possession of but a single petal, the standard. Moore (1936), on the basis of floral anatomy, verifies the corollar nature of this structure (not staminal in origin as in Petalostemon) and states that there are no vestiges of vascular traces for the other petals. Possibly the genus represents an intermediate stage in an evolutionary sequence of corolla reduction terminating in Parryella, which is apetalous.

The species of Amorpha have been twice summarized in the past forty years: Rydberg (1919-20) and Palmer (1931). In connection with the description of a new species, Wilbur (1954) presents a key to the southeastern dwarf kinds.

Eysenhardtia HBK. Nov. Gen. and Sp. 6:489. 1824.

Shrubs or trees. Leaves odd-pinnate, mostly with

numerous leaflets. Stipules semi-persistent or caducous, triangular to filiform. Stipels present but inconspicuous, subtending leaflets. Flowers in slender spike-like racemes, these terminal or axillary. Bracts early deciduous. Calyx nearly regular or decidedly irregular. Corolla white, scarcely papilionaceous. Petals five, free from each other and androecium, similar in appearance but standard slightly larger. Stamens 10, free for most of length. Pods well exserted, straight or somewhat falcate, usually thin-walled, mostly with a single seed.

Perhaps 10 species, southwestern United States to Guatemala, mostly Mexican, of arid areas. Our 1 or 2 species range from southwestern Texas to southern Arizona.

Psoralea L. Sp. Pl. 762. 1753.

Perennial herbs (ours) from woody or fleshy taproots or spreading rootstocks. Habit diverse, most species with branched, ascending or erect stems, some subscapose. Leaves mostly 3–5 (7) palmately-foliolate, some pinnately 3-foliolate, or upper leaflets unifoliate. Stipules present, usually persistent, triangular to acicular. Stipels not seen. Inflorescences raceme or spike-like, usually on well-developed axillary peduncles. Flowers scattered or congested, short-pedicelled to subsessile, usually 2–3 at each node in axil of a bract. Bracts various, semi-persistent or caducous. Bracteoles apparently not present. Calyx nearly regular or two-lipped, sometimes obliquely gibbous at base. Teeth very short or well-developed, lanceolate or semi-foliaceous, the lower usually the longest, upper two partially fused for part of length. Petals all present, papilionaceous, free from stamen tube, usually bluish, lavender to white, distinctly clawed, auriculate or lobed at base. Keel and wings asymmetric, lower portions often adherent. Keel frequently dark-tipped. Stamens diadelphous; fused filaments joined almost to tip; free stamen often shorter than others and apparently sometimes absent (stamens then monadelphous). Style usually abruptly bent or curved upwards at distal end. Fruit exserted from persistent calyx or nearly enclosed, the body roughly ovoid, often conspicuously beaked, compressed or turgid, 1-seeded. Pericarp covering various, thick-walled or bony to thin-membranous, ridged, reticulate or warty to nearly smooth, usually glandular. It is at maturity indehiscent, or may in some species fragment irregularly, or in a somewhat circumscissile fashion.

A large (over 100 species) and widely distributed genus, chiefly

American and African, some Australian. Species occur in most parts of the United States except for the northeastern provinces.

This genus has been segregated into several smaller groups by Rydberg (1919-20, 1928). Very few subssquent authors have followed Rydberg's interpretations; MacBride (1922) has strongly advocated the traditional circumscription.

Psoralea is indeed diverse. Possibly it consists of several phyletic units, but these do not appear as clearly discrete as in Dalea. Even the African woody species seem closely coherent with our North American types as regards total flower and fruit characters. Rydberg's segregates are based primarily on pod diversity which does not appear to correlate satisfactorily with variation otherwise, or distributional peculiarities.

Rydberg (1928) emphasizes the character of dehiscence in some of his groups, a point of some interest since the pod is basically indehiscent in the Psoraleae. It is questionable whether the term dehiscent is properly employed; certainly it is not typical legume sutural dehiscence. In species which possess a thin, membranous pericarp (especially Rydberg's Pediomelum), there is a tendency for the fruit coat to crack or split at maturity, partially exposing or releasing the seed. Sometimes this breaking is transverse near the apex and emulates circumscissile dehiscence. It seems likely that the factors involved are the increasing brittleness of the thin pericarp as it drys, and pressure exerted by the enlarging seed. If this behavior can properly be called dehiscence, it is a secondarily derived tendency among the species involved.

Rydberg's (1919 - 20) contribution is the only reasonably modern treatment of Psoralea in the United States. One must otherwise turn to state or regional floras for the identification of species. There are perhaps more species o Psoralea in Texas than any other state; these have recently been treated by Shinners (1951).

Dalea Juss. Gen. 355. 1789. (Parosela Cav. Descr. Pl. 185. 1802).

Annual or perennial herbs or shrubs of diverse habit, sometimes spiny. Leaves odd-pinnate with several leaflets, a few with three leaflets or simple. Stipules usually present but small and often early deciduous. Stipels present in some, ephemeral or gland-like. Inflorescence diverse, racemes or spikes, terminal or axillary, compact or loosely flowered. Bracts narrow and inconspicuous to broad and clasping, early deciduous to persistent; bracteoles present in some species-groups. Calyx 5-toothed, the lobes short or long (exceeding petals). Petals various in color, papilionaceous in appearance. Standard long-clawed, inserted on hypanthium, usually broad, often shorter than other petals. Wings and keel oblique and usually auricled, variously positioned, arising from hypanthium or from stamen tube (lower portion to near top); keel often the largest. Stamens monadelphous, 9 or 10 in species examined (said to range from 8—10); pod one-seeded, usually thin, indehiscent, included, or exserted in some woody species.

A large American genus including perhaps 150 species, United States to Chile, mostly arid regions and mountains, especially abundant in Mexico. The principal concentration of our kinds is in the southwestern states, a few extending east of the Mississippi.

There has been some tinkering with the generic delimitation of Dalea—a common phenomenon when large and polymorphic genera are involved. Rydberg (1919-20, 1928) characteristically split the group into a number of segregates. Shinners (1949) has recently gone to the other extreme, merging Dalea and Petalostemon. Wiggans (1940) and Kearney and Peebles (1951) have taken intermediate positions.

Rydberg's generic concepts in this group have not been taken up by most subsequent authors. However, they may have more merit than this evaluation of posterity would seem to imply. Essentially Rydberg removed from the main Dalea-plexus (Parosela of his treatment) three small woody groups in which the petals are all free from the stamen tube (Psorobatus Rydb., Psorodendron Rydb., and Psorothamnus Rydb.), and one, Thornbera Rydb., in which the petals were said to arise from the apex of the stamen tube. Segregation of the former groups may not be illogical, although Psorodendron and Psorothamnus might best be combined. The generic distinction of Thornbera appears weak. Rydberg (1919-20, 1928) states that the petals other than the standard are inserted at the mouth of the staminal tube as in Petalostemon. This does not always seem to be the case. I have examined material of several species which Rydberg has transferred to Thornbera. In some, it is true, insertion of the petals appears to be terminal on the stamen tube. In others, the petals while arising near the apex of the androecial sheath, are lateral as in other species of Dalea. Apparently, Rydberg eventually made similar observations for he notes in his latter publication (1928), and somewhat in conflict with his diagnosis of the genus, that the "filaments are free above the insertion of the petals." Since petal attachment is characteristically diverse in Dalea, it would appear that Thornbera may not have too much to stand on.

With respect to the coalescence of Petalostemon and Dalea, Shinners (1949) notes that Thornbera is intermediate between the two groups, thus providing evidence of their close relationship and calls attention to the long-clawed keel petals of Petalostemon multiflorum in Texas. In regard to this latter point, Shinners was apparently considering a reduced claw to be characteristic of Petalostemon, a long one definitive for Dalea—as stated in some diagnoses. This is true in a general way, but claw length is quite variable in Petalostemon and comparison with the often longer claws of Dalea has only descriptive, not diagnostic value. Thornbera, as indicated by the above discussion, is probably not a valid link between Petalostemon and Dalea. Furthermore, the nonpapilionaceous "corolla" of Petalostemon is comprised of modified stamens—there are no true petals—while Dalea apparently has a normal papilionaceous corolla.

Dalea is, then, herein interpreted in the broad sense, but exclusive of Petalostemon. Rydberg's segregation of the free-petal elements is not rejected on the basis that it is unreasonable. In aggregate genera of this type, the best point at which to draw generic lines is in some instances clearly definitive, but in others may be subjective (i.e., taxonomic judgment), or a matter of convenience. One item of convenience

is that of adhering to the usual generic lines unless they are manifestly untenable.

The use of Parosela Cav. for this genus by Rydberg and other authors is based on the fact that Dalea Juss. (1789) is antedated by Dalea Mill. (1854). Dalea Juss. is now conserved.

Major studies of United States species of Dalea include: (1) Rydberg (1919-20). All North American species are treated. The keys are difficult to use and specific lines are often tenuous. (2) Kearney and Peebles (1951). Arizona species only are included, but they constitute the majority of United States representatives. (3) Wiggans (1940). Although concerned with representatives of the Sonoran Desert in Mexico, the excellent keys and illustrations include many southwestern United States species. (4) Tidestrom and Kittell (1941), Arizona and New Mexico species.

Petalostemon Michx. Fl. Bor. Am. 2:48. 1803. (Kuhnistera Lam. Enc. 3:370. 1789). Prairie clover.

Herbs, usually perennial and arising from long-lived woody caudices, less frequently from slender rootstocks. Stems clustered, often numerous, mostly erect or ascending, simple below, usually branched above. Leaves odd-pinnate with 5—7 leaflets in majority of species, frequently reduced at apex of stems. Leaflets elliptic to linear, sometimes narrowly involute. Stipules setaceous, usually falling. Stipels suggested by glandular protuberances contiguous to leaflets. Flowers in dense, cylindric or conical, sometimes ovoid spikes, these terminating main stem and leafy branches. Bracts early deciduous, approximating length of calyx, obovoid to lanceolate, usually broadest near middle and tapering abruptly to a cusp or awn-like tip. Filiform bracteoles present in some species (P. candidum (Willd.) Michx. and relatives), apparently arising underneath calyx, persistent on rachis after abscission of flowers. Calyx somewhat asymmetric, short-toothed in the majority of species. True corolla lacking, replaced by nonpapilionaceous modified stamens. Dorsal "petal" (in position of standard) long-clawed, arising from hypanthium, usually broadened or cordate at base. Remaining petaloid structures, four in number, arising at apex of stamen tube, alternating with functional stamens, strap-shaped to somewhat asymmetric, narrowed at base. Stamens 5, monadelphous; tube approximating or slightly shorter than calyx. Pod included to slightly exserted, indehiscent, 1—(2) seeded. Stylar beak offset to standard (adaxial) side of calyx; at maturity, entire pod curved in this direction.

An American genus of possibly 35 species; Rydberg (1919-20) recognizes 45 kinds in the group as herein circumscribed, but some of these

should be reduced. These range from northern Mexico throughout much of the United States north to Saskatchewan. The greatest number of species appears to be in Texas. Another complex is to be found in the southeastern states. A smaller number of more widely distributed kinds (e.g., P. purpureum (Vent.) Rydb., P. candidum (Willd.) Michx., P. occidentale (Heller) Fern., P. villosum Nutt.) extend the genus throughout the Mississippi Valley and adjacent provinces. The principal areas in which Petalostemon is essentially absent are: (1) the Pacific Coast states, but one species in eastern Washington and Oregon, and another extending to the Mohave Desert in California, and (2) the northeastern deciduous forest, except for numerous (recent?) incursions from the West.

As circumscribed both by technical characters and rather uniform habit, Petalostemon is a well-defined genus. Closest affinities would seem to be with the polymorphic Dalea, some members of which closely resemble Petalostemon in general appearance. Problems relating to the presumed juxtaposition of the two are discussed under the former.

In the southeastern states there are 2—3 closely related species with considerably enlarged and persistent floral bracts, the outermost of which are sterile. These have sometimes been segregated as Kuhnistera Lam. The floral structure is, however, precisely that of Petalostemon.

The proper interpretation of the Petalostemon flower has been a matter of some doubt. Historically, the majority of workers have assumed that the keel and wing petals were connivent with the stamen column, thus arising at the orifice of that structure. Both Gleason (1952) and Porter (1957) have taken the position that the true corolla consists of the standard only, the remaining petaloid structures being staminodes. The diagnosis presented in this paper (that all of the petals are lacking) is based upon the interpretations of floral anatomy (Moore, 1936, working with material of P. candidum). This author shows that only the calyx arises from the usual perianth traces; his conclusion that the corolla cycle has been lost seems reasonable and, furthermore, is consistent with external appearance of the androecial "petals" which bear little resemblance to the ordinary papilionaceous corolla. What, however, of the presumed relationship of Petalostemon and Dalea? Other characters, including chromosome number, suggest a moderate degree of association between the two groups, but there seems little reason to question the petaloid origin of the papilionaceous corolla of the latter genus. Furthermore, there is a peculiarity in Moore's observations on Petalostemon. He shows the stamen traces to arise in two groups of 5 each, rather than in a single set which seems to be characteristic of other leguminous flowers studied. The set which gives rise to the petaloid structures arises immediately contiguous to the perianth whorl which produces sepals only. If two organs ordinarily of separate morphological origin become structurally associated, one wonders if any clear-cut differences can be properly attributed to them. Possibly, the petaloid structures in Petalostemon cannot be properly said to be fundamentally either stamens or petals, but arise from growth points having some physiological attributes of both.

Kuhnistera Lam., published in 1789, was employed by the majority of American code authors in lieu of Petalostemon Michx. (1803), and

thus appears in several of our older manuals—the alternative use of *Kuhnistera* in addition to *Petalostemon* for taxonomic reasons has previously been discussed. *Petalostemon* has now been conserved over *Kuhnistera* in the sense that the two are taxonomically to be considered synonymous.

The noun *Petalostemon* has been subject to alternative spellings and interpretations of gender (masculine and neuter). The above is that adopted in the *Nomina Generica Conservanda*.

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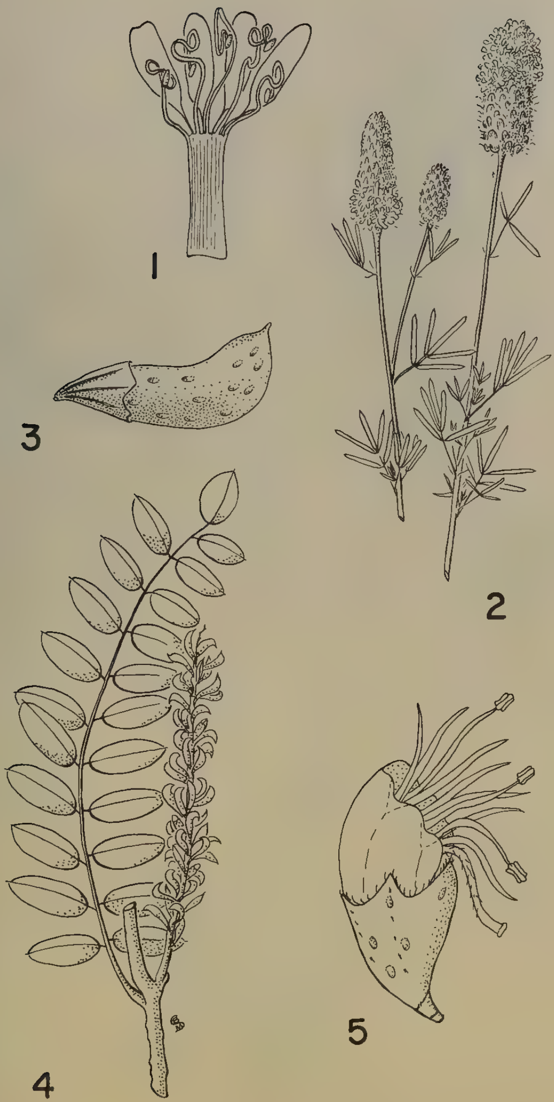

Plate I. Petalostemon purpureum. 1. Stamen sheath (opened and spread) and attached "petals" x6. 2. Inflorescence x2/3. Amorpha fruticosa. 3. Pod x4. 4. Leaf and fruiting spike x2/3. 5. Flower (late anthesis) x7.

Plate II. Psoralea psoralioides. 1. Leaf x1. 2. Fruit x3.
Psoralea tenuiflora. 3. fruit x4. 4. Habit x1.

Plate III. *Dalea enneandra*. 1. Habit x1 1/2. 2. Flower (open and spread) to show petal attachment x6. *Eysenhardtia polystachya*. 3. Pod x2. 4. Flowering branch x1 1/3.

Plate IV. Parryella filifolia. 1. Leaves and flowering branch x2/3. 2. Fruits xl 1/3. 3. Flower x6.

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A STUDY OF THE WEIGHT OF THE CORPUS LUTEUM DURING PREGNANCY IN THE COW[1]

R.M. Melampy and D.G. Gay

Department of Animal Husbandry
Iowa Agricultural and Home Economics Experiment Station
Iowa State College, Ames

Several investigators have demonstrated the importance of a functional corpus luteum as well as the hormone progesterone during pregnancy in the cow. Hess (1921) and Schmaltz (1921) have stated that the removal of the corpus luteum is followed by abortion in the pregnant cow. Hammond (1927) observed that the corpus luteum maintains its size and shows no signs of involution during gestation. Furthermore, since a cow does not come into estrus for about 30 days after parturition, Hammond (1927) concluded that the atrophy of the corpus luteum is delayed until that time.

The progesterone requirements for embryonic survival in the cow have been studied by Raeside and Turner (1950). They reported that daily subcutaneous injection of 25 mg of hormone did not maintain pregnancy in dairy heifers in which corpora lutea were removed in early stages of gestation. In an experiment in which the daily dosage was 50 mg for a period of 100 days, two out of four heifers had living fetuses at mid term. Uren and Raeside (1951) removed corpora lutea from three animals in advanced gestation and administered no progesterone. One animal gave birth to a calf on the 273rd day of pregnancy, one aborted and one died of a ruptured uterus. McDonald et al. (1952) reported that cows aborted in the absence of progesterone replacement therapy if the corpora lutea were removed between the 92nd and 163rd day of gestation. Pregnancy was maintained by injection of 100 mg of progesterone daily in cows in which corpora lutea were removed at approximately the 60th day of pregnancy. In animals in which the corpora lutea had been removed, abortion did not occur when the hormone injections were discontinued as early as the 162nd day of gestation. McDonald et al. (1953) also found that cows maintained early pregnancies after corpus luteum ablation when aqueous suspensions of 500 mg of progesterone were given at 10-day intervals. The purpose of this study is to evaluate the weight of the bovine corpus luteum in terms of its functional significance during pregnancy.

MATERIAL AND METHODS

A total of 298 pregnant cows (Angus, Hereford, Holstein, and Shorthorn) were used to furnish material for an evaluation of the weight of the bovine ovary and corpus luteum during pregnancy. Data on the ages and live weights of these animals were not available. References to specific breeds are given with the qualification that the dams and fetuses are not

[1]Journal Paper No. J-3417 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project 1325.

necessarily purebreds and the sires of the fetuses are unknown. The reproductive tract was removed from each cow soon after the abdominal cavity was opened.

The estimated age of the corpora lutea of pregnancy was based on the crown-rump length of the fetus. This measurement was made according to Stoss (1944) and represents the distance in centimeters from the fontanel to the tuber ischium (Table 1). The combined weight was obtained of the ovary including the corpus luteum of pregnancy. In addition, the corpus luteum was dissected from the ovary and weighed.

Table 1. Age of fetus and crown-rump length (Stoss, 1944)

Age of fetus Months	Crown-rump length cm.
1	1.5
2	8
3	15
4	24
5	35
6	48
7	63
8	80
9	90
10	100

RESULTS

Data on the combined weight of the ovary and corpus luteum of pregnancy are summarized in Fig. 1. The weights of the corpora lutea are given in Fig. 2. Fig. 3 presents the relationship between the weight of the corpus luteum and the combined weight of the ovary and corpus luteum in the case of the Hereford breed. The mean weights of corpora lutea from various breeds and 95 per cent confidence intervals are given in Table 2.

Table 2. Average weights and confidence intervals of corpora lutea among breeds

Breed	No.	Mean (gms)	X^2	s^2	95 per cent confidence interval (gms)	99 per cent confidence interval (gms)
Angus	60	4.800	1441.30	0.990	4.54-5.08	4.45-5.15*
Hereford	132	4.730	3070.78	0.893	4.57-4.89	4.52-4.94
Shorthorn	60	5.220	1689.99	0.935	4.97-5.47	4.89-5.55
Holstein	27	5.402	822.54	1.335	4.95-5.86	4.79-6.02

DISCUSSION

The combined weight of the ovary and corpus luteum of pregnancy ranged from 6.0 to 13.0 gm in 90 per cent of the cows examined. Furthermore, approximately 8 per cent weighed more than 13.0 gm. The data in Fig. 1 compare favorably with those reported by Hammond (1927).

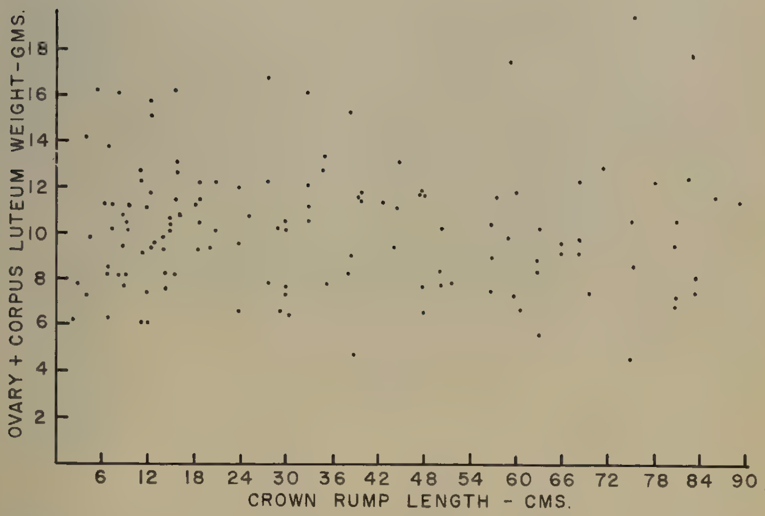

Fig. 1. Weight of the combined ovary and corpus luteum during pregnancy in the cow

According to the data in Fig. 2 there is no correlation between the corpus luteum weight and the stage of pregnancy. The weights ranged between 3.0 and 6.5 gm. Hammond (1927) observed no alteration in the ratio weight of ovary with corpus luteum due to the period of pregnancy, thus proving indirectly that the corpus luteum maintains its size throughout pregnancy. However, this ratio varies with the age of the animal, being greater in heifers than cows due to the accumulation of ovarian stroma in the latter. According to Hammond (1927) Bergmann has also presented data indicating that the bovine corpus luteum maintains a relatively constant weight throughout pregnancy. The size and structure of the corpora lutea remain unchanged during the second half of pregnancy in the rabbit (Hammond, 1917) and in the sow (Corner, 1915). Foley and Reece (1953) observed a definite, though by no means constant, increase in weight of the corpus luteum with advancing gestation up to the 45th day. Differences in age, breed, and individuality probably explain the variations that occurred. Foley and Reece (1953) also noted that the

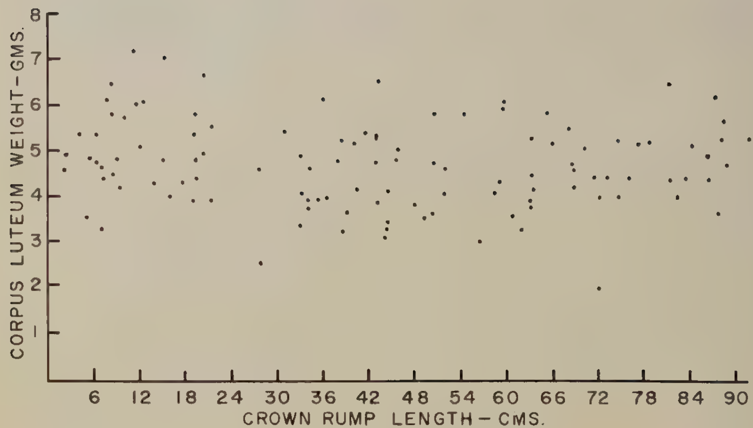

Fig. 2. Weight of the corpus luteum during pregnancy in the cow.

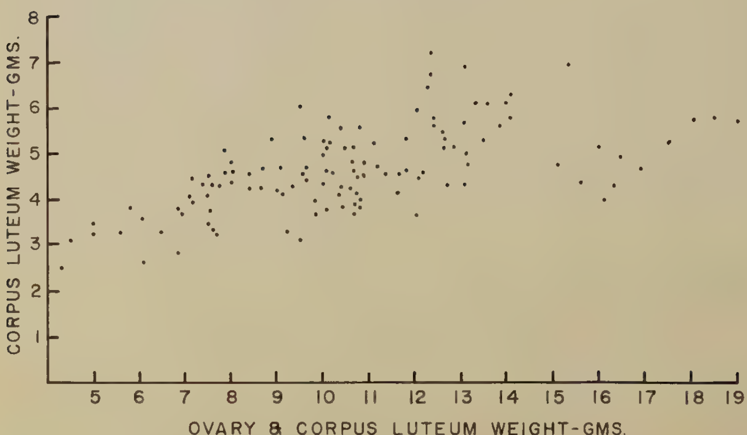

Fig. 3. Relationship between the weight of the corpus luteum and combined weight of the ovary and corpus luteum in Hereford cows.

sizes and weights of the corpora lutea of pregnancy are larger and heavier than those of the estrous cycle in the cow. The average weight of those from nonpregnant heifers was 3.27 gm which is 1.51 gm lighter than the average weight of the corpus luteum of pregnancy. In the case of the rat, Stafford *et al.* (1947) demonstrated a definite increase in the weight of the corpus luteum as gestation progressed.

The mean weights of the corpora lutea for the four breeds studied ranged from 4.8 gm in the case of the Angus to 5.4 gm for the Holstein (Table 2). The 95 per cent confidence intervals expressed in grams were quite similar for the Angus, Hereford, Shorthorn, and Holstein breeds. The greatest mean difference in weights was between Herefords and Holsteins. However, an analysis of variance of these data indicated the difference is not significant.

As is shown in Fig. 3 the corpus luteum accounted for approximately one-half of the combined weight of the ovary and corpus luteum when the latter was between 6 and 13 gm. However, when the total was greater than 13 gm the individual weights of the corpora lutea were approximately 6 gm. The data presented in Fig. 3 represent those for the Hereford breed. However, similar results were obtained on the other breeds studied. These observations suggest that the weight of corpus luteum of pregnancy in the cow is equivalent to about one-half of the combined weight of the ovary and the corpus luteum.

SUMMARY

A study was made of the weight of both the ovary and corpus luteum of pregnancy in the cow. The estimated age of the corpus luteum was based on the crown-rump length of the fetus.

In the 298 cows examined, 90 per cent of the ovaries, including the corpus luteum of pregnancy weighed between 6 and 13 gm. The weights of the corpora lutea ranged from 3.0 to 6.5 gm and were approximately one-half of the total ovarian weight. No relationship was observed between the weight of the ovary or the corpus luteum and the stage of pregnancy in the cow. The mean weights of the corpora lutea in grams were as follows: Angus, 4.80; Hereford, 4.73; Shorthorn, 5.22; and Holstein, 5.40. The mean weights of the corpora lutea for each breed were not significantly different.

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A COMPARISON OF DIFFERENT TYPES OF STALLS FOR DAIRY CATTLE[1]

C.F. Foreman, N.H. Curry, P.D. Homeyer, and A.R. Porter

The requirements for dairy cattle housing are changing rapidly over the United States. An increase in body size of cows and in number of cows per herd is a factor. This change has resulted in the need for remodeling existing dairy buildings or for new construction.

Considerable work with loose vs. conventional housing has been reported in the literature. Heizer et al. (2) have reported on a typical study comparing these two systems of housing lactating cows. Their work indicates little difference in milk production or in efficiency of production between the two groups.

However, many dairymen, particularly in the northern area of the United States, still prefer to winter cows in the more conventional stall-barns.

Ellington and Knott (1) compared four different types of stalls with reference to sanitation, labor, and bedding. They report that the daily bedding requirements per cow were 21.25, 4.7, 3.8, and 3.8 lb, for the box stall, stanchion, Hoard's, and modified Hoard's stall, respectively. The labor of cleaning and bedding in minutes per cow per day was: box stall 15.0, Hoard's stall 5.0, modified Hoard's stall 5.0, and stanchion 4.2. They found no difference in feeding or milking time or in the cleanliness of the milk produced.

Monroe et al. (4) report practically no effect of stall type on milk production where comparisons were made between box and tie stalls and between box stalls and stanchions.

Porterfield et al. (5) conducted a comparison of comfort and tie-chain stalls. They found that the cows in the comfort stalls produced more milk, sustained fewer injuries, were apparently more comfortable and remained cleaner than those in the tie-chain stalls. Essentially no difference in the amount of bedding used or in labor requirements was found.

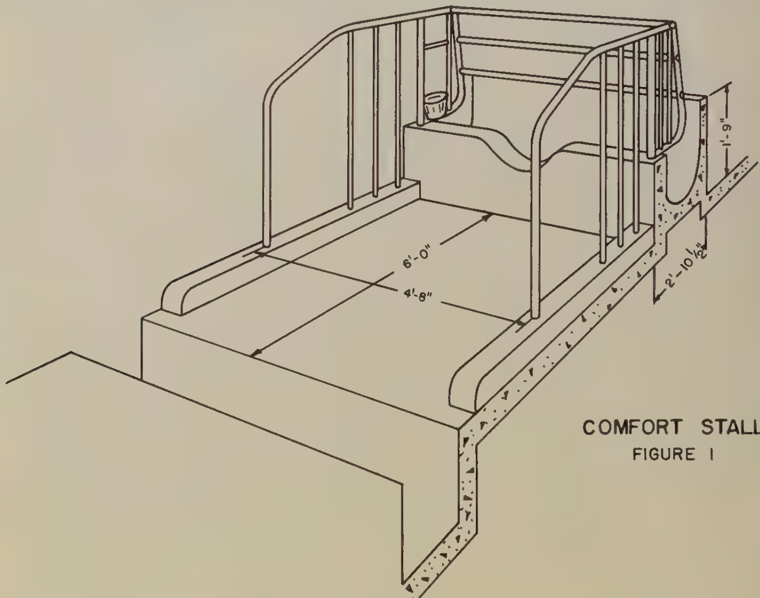
The purpose of an experiment at Iowa State College was to compare the three types of stalls—comfort, tie, and stanchion—which are most commonly found in conventional housing. Studies were made on cleanliness of stalls, cleanliness of cows, health of cows, bedding requirement, labor in cleaning and bedding, and in the apparent comfort of the cows.

Experimental Procedure

The experimental stalls were those in use in the Iowa State College dairy barn. The comfort-stall platforms were 72 in. long and 56 in.

[1]Journal Paper No. J-3419 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1292.

wide, and were equipped with an electric cow trainer, as described by Keepers (3). A sketch of this stall is shown in Fig. 1. A mechanical barn cleaner was used with these stalls.



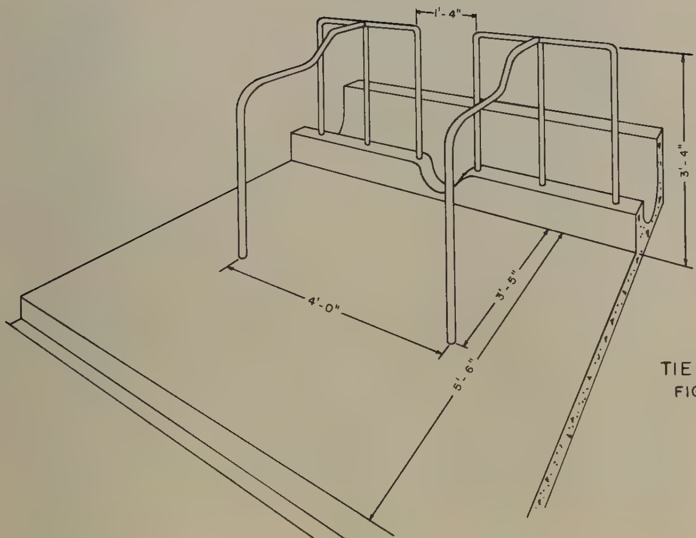
COMFORT STALL
FIGURE 1

The tie-stall platforms were 66 in. long and 48 in. wide as shown in Fig. 2. The stanchion platforms were 62 in. long and 46 in. wide as shown in Fig. 3. Both of these stalls were cleaned by hand using a manure spreader which was pulled through the barn. During the last three experimental periods an electric cow trainer similar to that used in the comfort stalls was used on the cows in the stanchions and tie stalls.

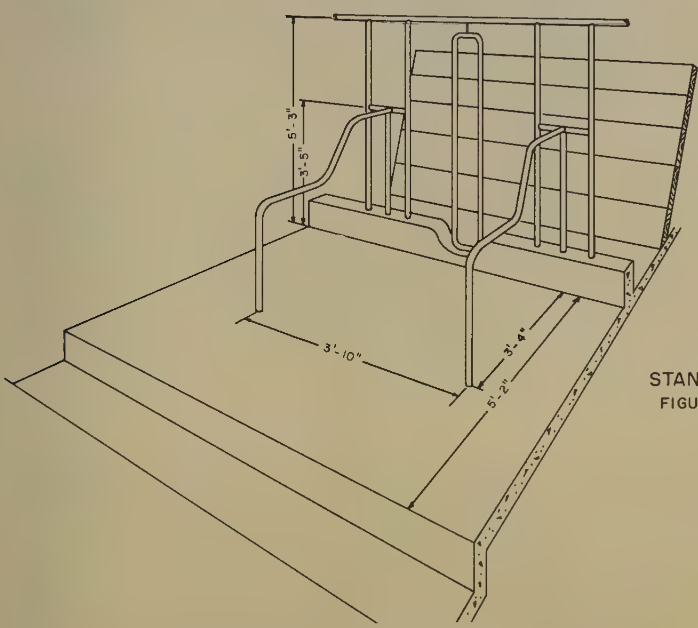
The data for this experiment were collected between January and April in 1956, using nine Brown Swiss and thirty-three Holstein cows. These forty-two cows were divided into fourteen groups of three each. One of each triad was stalled in a comfort stall, one in a tie stall and the third in a stanchion providing fourteen experimental animals in each type of stall. The cows in each group were selected according to size to fit the type of stall to which they were assigned. The members of each triad were grouped as closely as possible for stage of lactation and age.

Feeding and management were identical for each group and were kept as uniform as possible throughout the experimental period.

Data were collected the first three days of each of the six experimental periods, except that data on apparent comfort were collected over one continuous 14-day period. The cows were carefully examined at the start of the experiment for any bruises or injuries. All cows were



TIE STALL
FIGURE 2



STANCHION
FIGURE 3

carefully curried and brushed to remove manure and in some instances "spot-cleaned" at the start of each experimental period. Observations were made prior to each morning and afternoon milking on the cleanliness of cows and stalls.

Gutters were cleaned twice daily and bedded once each day following inspection. Time studies on all labor involved in cleaning gutters and stalls and in bedding were made with a stop watch. Records were also kept on the amount of bedding used on each stall.

The method of scoring cleanliness of cows and stalls is shown in Table I.

Table I. Grading system to measure cleanliness

Cow	
0	= No visible signs of manure or stain.
1	= Stained but no manure.
2	= Stained and/or manure on hocks or tail.
3	= Stained and/or manure on hocks and tail.
4	= Stained and/or manure on hocks, tail, and one flank.
5	= Stained and manure on hocks, tail, and both flanks.
6	= Manure on flanks and udder only.
Stall	
0	= No wet spots or manure on stall.
1	= Wet spots but no manure on stall.
2	= Manure but no wet spots on stall.
3	= Wet spots and manure on stall.

An attempt at evaluation of apparent comfort was made by recording the time reclining or standing as a measure of comfort. Fig. 4 shows the method of recording these data. Thermocouples attached to wires leading to a 16-point recording potentiometer were placed under metal strips laid on each stall platform. While the cows were standing a lower temperature was recorded than when the cows were lying on the thermocouples. The recording apparatus thus shows each change in position of the cow by a change in recorded temperature.

Experimental Results

The average scores indicating cleanliness of cows in the different stalls are shown in Table II. The comfort stalls were equipped with electric trainers throughout the experiment. Tie stalls and stanchions were equipped with these trainers only during the last three experimental periods. There was a statistically significant difference in the cleanliness of the cows in the different stalls during the first three experimental periods. The average cleanliness score for the comfort stall, tie stall, and stanchion was 1.03, 2.43, and 1.93, respectively, for the first three periods. This is in contrast to scores of 1.23 and .63 with the tie stalls

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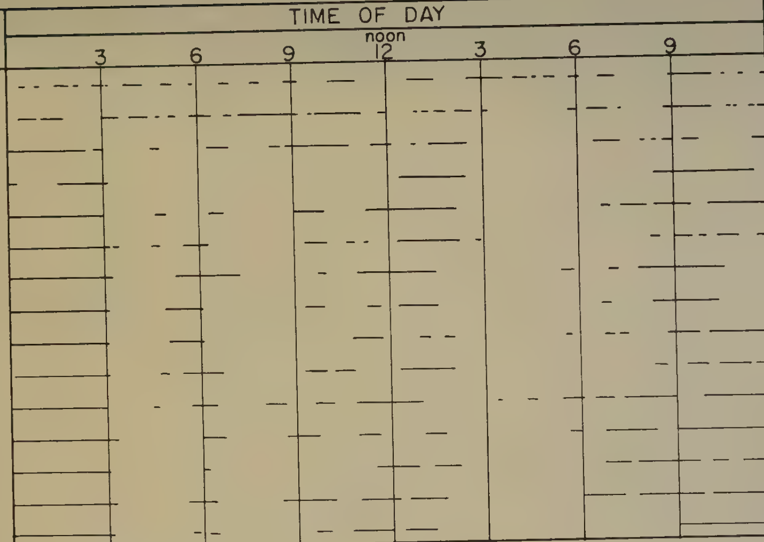

COW NO.	TIME (Hrs.) Day	TIME (Hrs.) Night	TIME (Hrs.) Total	TIMES Day	TIMES Night	TIMES Total
1	5.2	5.4	10.6	9	15	24
2	6.8	6.2	13.0	13	12	25
3	5.4	5.4	10.8	7	8	15
4	2.2	5.2	7.4	1	3	4
5	4.6	7.4	12.0	4	5	9
6	4.4	5.4	9.8	7	7	14
7	4.8	6.8	11.6	3	4	7
8	3.6	5.6	9.6	4	3	7
9	2.6	5.0	7.6	4	6	10
10	4.4	5.8	10.2	5	5	10
11	5.0	8.0	13.0	7	5	12
12	3.2	8.4	11.6	4	4	8
13	2.6	7.4	10.0	3	5	8
14	5.2	8.2	13.4	4	4	8
15	3.8	6.2	10.0	5	2	7

——— Cow is reclining

TIME RECLINING (TYPICAL DAY)

FIGURE 4

Table II. Average cleanliness of cows

Type of stall	Experimental period						Average*	
	1	2	3	4	5	6	First 3 periods	Last 3 periods
Comfort	2.1	0.7	0.3	0.5	0.6	0.5	1.03	0.53
Tie	2.7	2.3	2.3	1.2	1.4	1.1	2.43	1.23
Stanchion	2.5	1.9	1.4	1.2	0.5	0.2	1.93	0.63

*Cow trainers used on all cows during last three experimental periods.

and stanchions during the last three periods when the trainers were in use. This difference in cleanliness of cows on the same stalls resulting from use of the electric trainers is statistically significant.

The average score for cleanliness of stalls is shown in Table III. There was a marked improvement in cleanliness of stalls during the last half of the experiment as compared to that during the first half. The effect of the electric trainers with the tie stalls and stanchions is highly significant. The differences among stall types during the first three periods approached statistical significance, while during the last three periods they were not significant. A significant difference in the cleanliness of the stalls between the morning and afternoon inspections was noted.

The average daily scores for the first three periods as compared with the last three for the comfort stall, tie stall, and stanchion are 1.05-.84, 1.65-.79, 1,56-.71, respectively.

Numerous injuries were sustained during the course of the experiment. None was serious with the exception of an injured teat on each of three cows in the comfort stalls. A summary of the injuries sustained is shown in Table IV.

A definite, although not large, difference in bedding requirements for the three types of stalls was observed. The average daily requirement of 9.1, 8.2, and 7.6 lbs of wood shavings for the comfort, tie, and stanchion stalls is in direct proportion to the size of the stall and is apparently not a measure of stall type, as such. The daily bedding requirements in pounds is shown in Table V.

The time required to clean and bed the different types of stalls and to curry and brush the cows is shown in Table VI. A mechanical gutter cleaner was used in cleaning the comfort stalls so no time data are available. The average cleaning time of 20 seconds per tie stall and 23 seconds for each stanchion indicates little difference in cleaning time. However, considerable difference in time was required to bed the different type stalls. The average bedding time of 24 seconds for stanchions as compared to 10 seconds for tie stalls and 14 seconds for comfort stalls is probably due to the difference in type of construction.

Average, minimum, and maximum daily reclining time and average, minimum, and maximum daily number of times reclining are presented by individual animals in Table VI with these values again averaged by stall types. Day to day behavior of any one cow was reasonably consistent, but wide variability existed in the behavior of cows similarly stalled.

Table III. Average cleanliness of stalls

Type of stall	Experimental period[a]												First 3 periods[b]		Last 3 periods[c]		First 3 periods[d]	Last 3 periods[d]
	1		2		3		4		5		6							
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM		
Comfort	1.8	0.8	1.2	0.9	1.3	0.4	1.1	1.0	0.3	0.6	0.7	0.6	1.44	0.67	0.94	0.74	1.05	0.84
Tie	1.9	2.1	1.8	1.4	1.6	1.0	1.2	0.9	0.8	0.5	0.6	0.4	1.80	1.50	0.88	0.60	1.65	0.79
Stanchion	2.2	1.7	1.5	1.2	1.9	0.9	1.0	0.9	0.5	0.3	0.1	0.4	1.85	1.28	0.90	0.52	1.56	0.71

[a] Cow trainers used with all stalls during last three experimental periods.
[b] Difference between periods highly significant.
[c] Difference between stall types highly significant.
[d] Difference between stall types not significant.

Reclining patterns appeared to be influenced far more by habit or temperament of individual animals, or by possible location with respect to cross alleys or other external disturbances, than by type of stall.

Table IV. Injuries during 90-day experimental period

	Type of stall		
Type of injury	Comfort	Tie	Stanchion
Rough or bare hocks or knees	10	14	17
Cut hocks or knees	2	3	3
Swollen hocks or knees	6	5	11
Injured teats	3	--	--
Other bruises	2	1	--
Stiffness	2	--	--

Table V. Bedding requirements (shavings) in pounds

Type of	Experimental period						
stall	1	2	3	4	5	6	Average
Comfort	12.4	10.3	8.4	5.8	7.4	10.3	9.1
Tie	8.7	8.3	9.1	7.9	7.9	7.5	8.2
Stanchion	9.6	7.6	7.3	6.8	6.7	7.5	7.6

DISCUSSION

The greater cleanliness of cows in the comfort stalls compared to those in either the tie stall or stanchion is undoubtedly due to a combination of greater stall area and the use of electric trainers. The importance of the trainers is obvious when we compare the significantly different scores for the first three with those of the last three experimental periods. The same comparative results are obtained when the average scores for cleanliness of stalls are studied. The marked difference observed in cleanliness of the comfort stalls and the cows in these stalls between the first experimental period and subsequent periods may be because the cows had not yet become adjusted to the larger stalls. The reason for slightly cleaner cows and cleaner stalls in the stanshion group compared to the tie stalls when the electric trainers were in use is not immediately available. Perhaps it is because the narrower stalls with stanchions restrain the cows sufficiently so that the stall platforms are not dirtied.

These results suggest that cleaner stalls and the resulting cleaner cows are not because of stall size but rather a matter of training the cows to void their body wastes in the gutter behind each stall and not on the stall platform. However, if we consider the health and comfort of

Table VI. Average, minimum, and maximum daily reclining times and average, minimum, and maximum daily number of times reclining during a 14-day period

Cow and stall No.	Stall type	Reclining time per day (hrs)			No. times reclining per day		
		Avg.	Min.	Max.	Avg.	Min.	Max.
1	Comfort	11.7	9.8	13.2	20.7	11	28
2	"	11.5	8.6	14.2	18.8	12	27
3	"	10.8	5.2	13.8	16.0	9	21
4	"	9.1	6.8	12.0	3.9	3	5
5	"	12.3	9.8	13.6	8.5	6	12
Avg. all comfort stalls		11.1	7.9	13.4	13.6	8.2	18.6
6	Tie	12.0	9.8	15.0	9.0	7	14
7	"	9.5	6.3	13.0	9.3	7	16
8	"	11.9	9.2	13.8	8.1	5	11
9	"	10.2	4.0	12.2	7.3	2	11
10	"	13.6	10.2	15.2	7.5	5	10
Avg. all tie stalls		11.4	7.9	13.8	8.2	5.2	12.4
11	Stanchion	14.3	12.8	15.8	12.0	9	17
12	"	11.6	10.0	13.6	9.3	6	12
13	"	11.2	9.2	13.4	8.1	6	10
14	"	12.9	10.2	14.0	8.9	6	14
15	"	10.7	9.4	12.6	8.6	5	13
Avg. all stanchions		12.1	10.3	13.9	9.4	6.4	13.2

the cows the question of stall type is not as easily answered. An increase in rough or bare, swollen, and cut hocks or knees was noted as stall size decreased from comfort stall to tie stall to stanchion. This is undoubtedly due to the manner in which the cows are restrained and in the restrictions due to stall area.

The average age of the cows in the comfort stalls was greater than those in the other two groups probably accounting for all or part of the stiffness and injured teats observed in that group.

The time spent in cleaning gutters will vary little between stall types, and, as in the case of the comfort stalls, can be done concurrently with other work if mechanical cleaners are available. The differences in bedding time were caused by convenience of bedding the different types of stalls and not the results of stall size or bedding used.

The limited test on apparent comfort of cows in the three stall types as measured by time reclining and number of times reclining per 24 hours was inconclusive. A more elaborate test in which cows would be rotated both within and among the stall types until information was obtained on the behavior of each cow in each stall type and position might provide a basis for some conclusion on apparent comfort.

Table VII. Time study data in seconds per stall[a]

Work done	Type of stall	Experimental period 1	2	3	4	5	6	Avg.
Cleaning gutter	Comfort[b]	-	-	-	-	-	-	
	Tie	20	29	17	24	15	17	20
	Stanchion	29	23	21	24	21	21	23
Bedding	Comfort	16	17	13	17	10	10	14
	Tie	9	12	12	7	13	9	10
	Stanchion	14	10	10	16	16	12	24
Curry and brush[c]	Comfort	67	39	31	36	39	-	42
	Tie	49	51	44	39	39	-	44
	Stanchion	68	47	31	39	47	-	46

[a]Average per cow per day in seconds
[b]Mechanical cleaner—no time data
[c]Based on limited data

The comfort stalls occupy more room than the tie stalls and the tie stalls more room than stanchions. If cost is the deciding factor, and if electric trainers are used, the results of this study would indicate that the stanchion or tie stall would be most economical. However, as stall area increased the number of bruised, cut, rough, and swollen hocks and knees decreased. Over long periods of time this difference might increase in importance. In addition the cows in the comfort stalls are more attractively displayed and are easier to work around than those in the tie stalls and the same applies in comparing the tie stall with the stanchion. This is of much greater importance to the breeder of registered cattle than to the commercial dairyman. In this regard most commercial dairymen will probably go to the loose housing system rather than any of our conventional stall barns. A final consideration is that of milk production.

While no milk production comparisons were made in this study, Porterfield _et al_. (5) report that cows in comfort stalls produce more than those in tie stalls. The difference in construction costs for the larger stalls might be justified strictly on the basis of greater milk yield. In addition the cows can be kept slightly cleaner, are less liable to be injured and are more attractively presented to prospective purchasers of breeding stock. All this is at relatively little increase in labor and bedding costs.

Summary

A comparison of comfort, tie, and stanchion stalls for dairy cattle was made with fourteen cows in each type of stall. Observations were made on cleanliness of cow and stall, bedding and labor requirement, and health and apparent comfort.

When electric trainers were used, there was little difference in the cleanliness of cows or stalls between the three stall types. The bedding requirements, in wood shavings, were 9.1 lbs, 8.2 lbs, and 7.6 lbs for the comfort, tie, and stanchion stalls, respectively. More time was spent in bedding the stanchion stalls than either comfort or tie stalls. Time spent in cleaning gutters each day was 20 seconds per tie stall and 23 seconds per stanchion stall. The comfort stalls were cleaned mechanically.

The cows in the comfort stalls sustained less injuries of flanks, hocks and knees than those in the tie stalls and those in the tie stalls less then those in stanchions.

Variability of behavior of individual cows in apparent comfort tests appeared to be more influential on reclining habits than did type of stall.

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NUMBERS OF MATURE WALLEYES IN CLEAR LAKE, IOWA, 1952-3, AS ESTIMATED BY TAGGING[1]

Richard R. Whitney[2]

Department of Zoology and Entomology
Iowa State College, Ames

ABSTRACT

Numbered strap tags were placed on the premaxilla of 2,232 walleyes during the 1952 and 1953 spawning seasons. Since good mixing of tagged fish on the spawning grounds was demonstrated, the number of walleyes on the 2 areas was estimated as 12,776 in 1952 and 16,311 in 1953. An estimated 12,119 of the 1953 spawning population were males. A prolonged spawning season kept males on the grounds longer than usual. Male walleyes were found in the spawning area up to 30 days after tagging. They apparently are not limited to a "home" spawning area, however. After spawning, walleyes apparently move to more vegetated areas of the lake. Angler returns indicated a harvest of 15.7 per cent in 1952 and 6.3 per cent in 1953. Experimental gill nets and 2-inch (bar

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[2]Now Project Leader, Susquehanna River Investigations, Chesapeake Biological Laboratory, Aberdeen, Maryland. The author wishes to express his appreciation to Dr. Kenneth D. Carlander for guidance and assistance throughout the study, and for preparation of the manuscript for publication from material in the author's Ph.D. thesis. For assistance in the field, the author is indebted to Dr. and Mrs. Carlander and members of his fishery management class, 1953, who helped with the tagging under severe weather conditions, to Muhrl Linquist, E.T. Rose, the late J.Z. Stevens, and T. Moen of the State Conservation Commission, to the hatchery fishing crews, especially Rex Johannesen, Sam Arnold, and Dick Mott who helped both years, to Richard E. Hennemuth and Charles J. Di Costanzo of the Cooperative Research Unit, to Harold Suttcliffe who helped in many ways and generously extended his hospitality for several weeks during the summer of 1953, and to James Gilruth and members of the Clear Lake Chamber of Commerce whose efforts were largely responsible for the tag returns from fishermen. Special thanks are also due Dr. Boyd Walker for permission to complete the analyses and writing while the author was employed on the Salton Sea Investigations at the University of California. The author's wife, Mary Lou, gave much encouragement and help.

measure) gill nets also gave data for population estimates. The experimental nets apparently sampled fish over age group III in proportion to their abundance. Estimates by size groups or age groups permitted a better assessment of confidence limits since all sizes were not tagged in equal proportions. An estimated loss of 40 per cent of the tags placed on fish in 1952 occurred in the course of one year. The annual mortality from 1952 to 1953 was estimated as 29, 27, and 56 per cent for IV, V, and VI year fish respectively. It was possible to derive somewhat independent estimates which generally corroborated the population and mortality estimates. The best estimate for 1952 was 6,030 walleyes over 16 inches. For 1953 a similar figure was 4,870 over 16 inches or 30,820 over 12 inches, total length. The latter figure corresponds to 8.5 walleyes per acre.

INTRODUCTION

The fishes of Clear Lake have been under study by the Iowa Cooperative Fisheries Research Unit since 1941. The walleye, Stizostedion vitreum vitreum (Mitchill), is the most sought game fish in the lake, but fishing success for this species has varied considerably from year to year. The present study was aimed primarily at obtaining an estimate of the number and rate of harvest of walleyes in the lake.

Clear Lake in north central Iowa has an area of 3,643 acres and is 5 miles long and about 2 miles wide at the east end (Bailey and Harrison, 1945). The maximum depth is about 20 feet and only 15 per cent exceeds 15 feet in depth. The lake is eutrophic, with an abundant, varied warm-water fish population.

MATERIALS AND METHODS

Walleyes were tagged in the spring during the spawning runs of 1952 and 1953. Linen gill nets of 2 and 2.5 inch mesh (bar measure) were used in 1952. In 1953, nylon gill nets of 2 inch mesh (bar measure) were used. Experimental gill nets with equal lengths of 0.75, 1.0, 1.25, 1.5, and 2.0 inch mesh (bar measure) were used to some extent both years.

Netting operations were for the most part carried out from two field stations, Shady Beach and Bayside (see Fig. 1). In 1952 walleyes were also tagged and released at the hatchery. Both field stations are located on rocky shores which are major spawning areas of the walleye.

The tags used were numbered monel metal strap or ear tags. Most of the tags used were size 3, being 7/16 inch long and 1/8 inch wide when clenched, but a few larger tags size 18, 1 inch long and 1/4 inch wide, were used on the larger fish. The tags were used as jaw tags being clamped around the upper premaxilla in most cases. A few walleyes were tagged around the entire maxilla or on the lower jaw (the mandible).

Sampling to estimate the population was done in the summer both years using experimental and 2-inch mesh gill nets. Chapman (1954) has reviewed the methods of population estimation. In the present study both Schnabel and Petersen type estimates were made, the former using

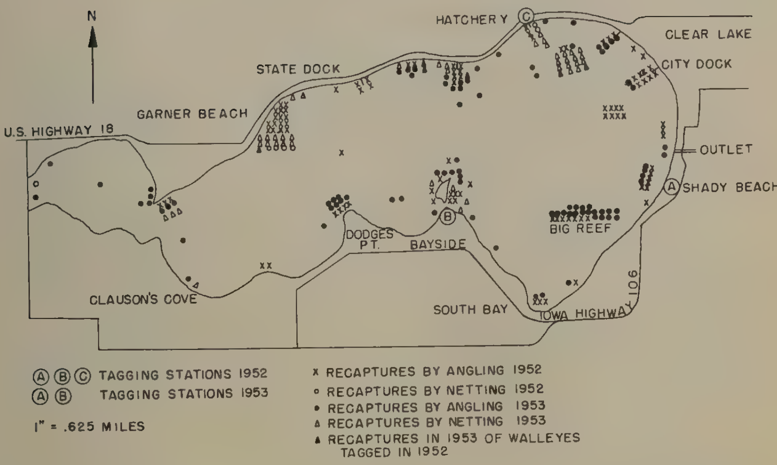

Fig. 1. Map of Clear Lake showing tagging and recapture locations.

recaptures made during tagging from the formula $N = \frac{Sn_tX_t}{Sx_t}$, and the latter from samples taken after tagging from the formula $N = X\frac{Sn}{Sx}$ where N is the population, n_t is the number of fish in the t^{th} sample, X_t is the total number of tagged fish in the population up to time t, and x_t is the number of tagged fish in the t^{th} sample. Confidence intervals were determined for the Schnabel estimates by substituting Poisson limits for Sx_t in the Schnabel formula, as suggested by DeLury (1951), and for the Petersen estimates by substituting binomial limits for $\frac{Sn}{Sx}$ in the Petersen formula. Tables from Ricker (1937) and Snedecor (1946) were used to determine the limits for the Poisson and binomial distributions respectively.

Tag returns from anglers, used in both years to estimate fishing success, were accomplished mainly by the generous cooperation of the Clear Lake Chamber of Commerce. Fishermen were encouraged to report tags to the Chamber of Commerce which held drawings for prizes at the end of the year. The tagging program was publicized in the local newspapers, and operators of boat concessions on the lake, all of whom were members of the Chamber of Commerce, checked the catches for tags as they were brought in. It is felt that these efforts were successful in bringing reports for a high percentage of the tagged fish captured by fishermen.

In conjunction with the tagging study, age and growth of the walleye were studied from scale samples collected in 1948 through 1953. The results will be reported in another paper.

TAGGING AND RESULTS IN 1952

The first tagging efforts were made from April 16 to April 28, 1952, during regular operations of the State Hatchery fishing crews. The crews operated at two field stations and most of the stripping and tagging were done at the stations. Fish which were not yet ripe, however, were taken by truck to the hatchery and held until ripe. They were then stripped and tagged at the hatchery. The method of procedure in tagging was to operate alternate nights at the two stations, tagging the fish as they were released. In addition, stripped fish were tagged each morning at the hatchery. In this manner 801 walleyes were tagged: 132 at the Bayside station, 161 at the Shady Beach station, 501 at the hatchery, and 7 during test gill netting not on the spawning grounds.

A small number of recaptures were made during the operations. The fishing crews cooperated by reporting such recaptures, but in most cases released the fish at the nets without recording the tag number, so that some confusion exists as to whether the fish had been tagged the same or some other night. At any rate, 23 recaptures were reported in a total catch of 1,371. A Schnabel-type estimate from these figures gives 12,776 walleyes with a 95 per cent confidence interval of 8,543 to 20,129. Obviously this estimate is not very reliable but it is in fair agreement with estimates subsequently made.

The Iowa State Conservation Commission carries on a creel census each year at Clear Lake. Their records indicated that 1,860 walleyes were censused in 1952. Since 126 tags were returned by fishermen, an estimate from these figures is 11,831 or a 95 per cent confidence interval of 8,207 to 14,053. The census was undoubtedly not complete though it may have been nearly so, and some fish were probably included in the census which were too small to have been tagged. If smaller fish were caught in proportion to their abundance, this estimate might be made to apply to the entire population of walleyes. It is doubtful that angling catches small fish in proportion to their abundance, though no evidence is available from the present study because no measurements were made of the fish censused.

From June 15, 1952 to September 6, 1952, experimental gill nets caught 557 walleyes of which 7 were tagged, to give an estimate of 61,615. It may be seen in Table 1, however, that the size distribution of the tagged population differs considerably from that of the population as represented by the experimental gill net catch. The estimate could still be made to apply to the entire population if it could be assumed that the experimental gill net captures fish of all sizes in proportion to their abundance.

If only walleyes over 16 inches in total length are considered, the numbers of each size group caught in the experimental gill net and in the tagged population are reasonably close. A chi-square test (Table 2) indicates no significant difference between the two. This implies that the two samples at least refer to the same segment of the population. An estimate referring to fish over 16 inches in total length at the time of tagging would therefore be valid. A total of 767 walleyes over 16 inches in total length were tagged, and experimental gill nets caught 55 fish, 7 of them tagged, which were 16 inches or more in total length at the

Table 1. Number of walleyes, by total lengths, in the 1952 experimental gill net sample compared to the tagged population and to recaptures

	Total length in inches											
	8-9.9	10-11.9	12-13.9	14-15.9	16-17.9	18-19.9	20-21.9	22-23.9	24-25.9	26-27.9	28-29.9	Total
Exp. gill net sample:												
As of time of capture	10	139	216	65	36	25	19	7	1	1	0	519[a]
As of last annulus[b]	187	200	43	36	17	15	16	4	0	1	0	519
Tagged population:	4	2	3	18	261	250	165	69	14	5	3	794[c]
Males	0	1	2	16	254	182	42	3	0	0	0	500
Females	0	0	0	1	7	66	122	64	13	5	3	281
Recapture in exp. gill nets	0	0	0	0	1	4	1	1	0	0	0	7

[a] Thirty-eight fish were not measured.
[b] Estimated from a subsample of 236 fish.
[c] Seven fish were not measured when tagged and sex was not determined on 13 specimens.

Table 2. Chi-square test to find whether the experimental gill net catch and the tagged population differ in sizes represented over 16 inches total length, walleyes, Clear Lake, 1952

	Length group				
	16-17.9	18-19.9	20-21.9	22-29.9	Total
No. of fish in exp. gill net[a]	17	15	16	5	53
No. of fish in tagged population	261	250	165	91	767
Total number	278	265	181	96	820
Expected proportion	.3390	.3231	.2207	.1170	

Chi-square = 2.24, non significant, 3 degree of freedom

[a]The lengths used are those at the last annulus rather than the lengths at capture, since the lengths at annulus formation are comparable to lengths at time of tagging.

time of tagging. An estimate of 6,030 walleyes over 16 inches in total length may be derived from the preceding figures. A 95 per cent confidence interval places the population between 2,984 and 13,456.

The small number of recaptures in 1952 eliminates the possibility of any further subdivision of the estimate so as to test the assumption that experimental gill nets catch all sizes of walleyes in proportion to their abundance. Evidence from 1953 which bears on this problem will be discussed in the next section.

TAGGING AND RESULTS IN 1953

Since the State Conservation Commission did not operate the walleye hatchery at Clear Lake in 1953, it was not possible to proceed in the same menner as in 1952. It was necessary to employ a crew to assist with gill nets. The fish were tagged and released with no stripping of eggs and sperm. It was desired to spend alternate nights as in 1952 at Bayside and Shady Beach. However, frequent high winds made it necessary to fish more often at the island near Bayside where a sheltered side was always available. A total of 1,431 walleyes were tagged in the interval from March 25, 1953 to May 15, 1953. The extended period of the spawning run was due to an early thaw followed by cold weather which may have delayed maturation. Dr. Kenneth D. Carlander, Mrs. Carlander, and members of his fishery management class tagged 292 walleyes at the island in the interval from March 25 to April 4, 1953. In the next 7 weeks 637 walleyes were tagged at the island, 443 in the

Shady Beach vicinity, 46 at Dodge's Point, and 13 not on the spawning grounds. During the tagging, 61 recaptures were made, leading to a Schnabel-type estimate of 16,311 walleyes with a 95 per cent confidence interval of 12,707 to 20,947.

The fact that only 142 of the walleyes tagged were females raises a question as to whether this estimate refers to the entire spawning population or only to the males and the few females which seem to have ripened during the cold weather. Recaptures on the spawning grounds included 4 males more than 30 days after they were tagged. Recaptures of 12 males were made on the spawning grounds 20 days or more after they were tagged. This provides evidence that the males tend to remain on the spawning grounds throughout the season. Only 2 recaptures of females took place, whereas 59 recaptures were made of males. Considering the ratio of the numbers recaptured to the total number tagged of each sex, it might be inferred that since the ratio for females is only about one-third that of males, the females tended to remain on the spawning grounds for a shorter time. Some upward distortion of the Schnabel estimate would result. An estimate applying only to males might be more appropriate from these data.

Some movement of the males took place between the spawning sites sampled (Table 3). Since about twice as many walleyes were tagged at the Bayside station as at the Shady Beach station, the same ratio would be expected to hold true in recaptures made at either station, provided that the assumption is met that the fish move randomly on the spawning grounds. Included in Table 3 then is a chi-square test for each of the stations. Since no significant difference exists between the ratios as tagged and as recaptured, it appears that good mixing took place in the lake. It is particularly interesting that 8 of the 13 fish recaptured at Shady Beach had been tagged at Bayside.

It is possible that the distance from Bayside to Shady Beach, approximately 1.75 miles, may be travelled by a fish during one night. It is more likely that as daylight approaches the fish retreat to deeper water (see Eschmeyer, 1950). With the onset of darkness they are again attracted to the shallow spawning areas and possibly move randomly in deep water until some stimulus directs them to a particular shore. Further evidence on this point comes from the fact that in 1952, 14 walleyes released at the hatchery (1.4 and 1.8 miles from the spawning areas) were later caught on the spawning grounds, 3 of them within 1 day and 4 within 2 days of their release. Eschmeyer (1950, p.37) felt that walleyes in Lake Gogebic, Michigan, revisited identical areas or tended to remain in restricted areas for a number of days during the spawning season. The evidence in Clear Lake is that there is no apparent choice of grounds by particular fish, revisits occurring no oftener than expected by chance.

A population estimate referring only to males should be quite reliable in view of the preceding discussion. Such an estimate using only the data from April 4 to May 1, 1953 when sex was easily determined, involves a total of 983 tagged male walleyes and 37 recaptures of male fish tagged in the same period. A Schnabel-type estimate gives 12,119 males on the spawning grounds with a 95 per cent confidence interval of 8,790 to 17,242.

Table 3. Chi-square tests to discover whether walleyes moved randomly between tagging stations, 1953

	Tagged at Bayside	Tagged at Shady Beach	Total
Total	929	443	1372
Expected proportion	.6771	.3229	1.0000
Recaptured at Shady Beach	8	5	13
Recaptured at Bayside	23	5	28
Total recaptured	31	10	41

Chi-square to test ratios of recaptures at Shady Beach

$$\frac{(8-8.80)^2}{8.80} + \frac{(5-4.60)^2}{4.60} = 0.22, \text{ non significant, 1 degree of freedom}$$

Chi-square to test ratios of recaptures at Bayside

$$\frac{(23-18.96)^2}{18.96} + \frac{(5-9.04)^2}{9.04} = 2.66, \text{ non significant, 1 degree of freedom}$$

In an attempt to obtain a Petersen-type estimate, the same nets used in tagging were set in the interval from June 29 to July 19, 1953. A total of 245 walleyes were caught, 27 of which were tagged, giving an estimate of 12,985 with a 95 per cent confidence interval of 10,624 to 16,695. There are several reasons why this estimate might be expected to differ from the Schnabel. Possible distortion of the Schnabel due to emigration of females has already been mentioned. The summer netting included all walleyes susceptible to capture in such nets and did not depend on their sexual maturity as did the spring sample.

A second estimate is available from walleyes captured in experimental gill nets operated from June 16 to August 28, 1953. A total of 236 fish were caught, of which 12 were tagged, to give an estimate of 28,169 with a 95 per cent confidence interval of 18,779 to 37,559. As in 1952, however, the size distribution of walleyes in the experimental gill net differs considerably from that of the tagged population. This point will be discussed in detail in the following section.

An attempt to arrive at an estimate from a creel census in 1953 did not prove particularly fruitful because of poor angling success.

The author encountered only 66 walleyes, 5 of which were tagged. Six of the 66 were 8 inches or less in total length. If these 6 are disregarded, the estimate of 17,179 is seen to agree in general magnitude with those of the Schnabel and the 2-inch mesh Petersen estimates.

COMBINATION OF 1953 SUMMER GILL NET SAMPLES

Since experimental gill nets and 2-inch mesh gill nets were both used during the summer of 1953 to obtain samples for Petersen type population estimates, it would be desirable to combine these samples to obtain one estimate. The effect would be to derive an estimate with narrower confidence limits because for a given proportion of tagged fish in the population, the binomial distribution gives more precise limits with an increase in the absolute number of tags in the sample. In order to combine the samples it is essential that they refer to the same segment of the walleye population.

Discussion of differences between the samples

Apparently the estimate from 2-inch mesh (bar measure) gill nets does not refer to the same segment of the walleye population as the estimate from experimental gill net (Table 4). In an attempt to reconcile the two estimates, Table 5 was compiled listing the sizes tagged and sampled. Naturally, fish sampled during the summer will have grown since the time of tagging, so that it is necessary to consider the length of each fish at the last annulus. This amounts to an approximate correction back to the time of spawning, most fish at that time showing evidence of an annulus at the outer edge of the scale. It would seem reasonable to consider only fish over 14 inches in total length in the samples. This eliminates only 1 tag from the experimental gill net catches. A chi-square test as shown in Table 6 does not now indicate that the samples might be from different populations.

Table 4. Comparison of the numbers of tagged and untagged walleyes in two samples, 1953

	Number tagged	Number untagged	Total
Exp. gill net	12	224	236
2" mesh gill net	27	218	245
Total	39	442	481
Expected proportion	.0791	.9209	

Chi-square = 5.95; significant, 1 degree of freedom

Unfortunately this is only a superficial test as a closer examination of Table 5 will reveal. In comparing the numbers tagged and subsequently sampled with the same 2-inch mesh nets, it may be seen that the summer sample contains too few small fish. This could be due to the fact that the nets were cut to remove the fish. The nets may have become progressively less efficient at catching small fish and more efficient at catching large fish. This could also be due to the fact that more females

Table 5. Total number of walleyes, by size groups, tagged in 1953 compared to numbers sampled in experimental and 2 inch mesh gill nets

	Total length in inches										
	6-11.9	12-13.9	14-15.9	16-17.9	18-19.9	20-21.9	22-23.9	24-25.9	26-27.9	28-29.9	Total
Tagged population	0	15	581	444	295	63	13	8	2	1	1422[c]
Untagged fish in exp. gill net[a]	43	52	84	30	10	1	3	1	0	0	224
Untagged fish in 2 inch mesh[a]	0	6	61	69	39	14	17	7	1	1	215[b]

[a]Lengths given are as of the last annulus which corresponds to the size at time of tagging.
[b]Length at last annulus not determinable for 3 fish.
[c]Nine fish were not measured when tagged.

Table 6. Comparison of numbers of tagged and untagged walleyes over 14 inches in total length in experimental and 2-inch mesh gill net

	No. tagged	No. untagged	Total
Experimental gill net	11	129	140
2-inch gill net	27	212	239
Total	38	341	379
Expected proportion	.1002	.8997	

Chi-square = .79; non significant, 1 degree of freedom

were available for sampling in the summer. This would have no effect on the population estimate if the same percentage of each size group had been tagged, or, in other words, if the fish had been tagged in proportion to their abundance.

A statistical test can shed some light on the question as to whether the fish of various sizes were tagged in proportion to their abundance. The frequency distribution of sizes of tagged fish should agree with that of untagged fish in the samples. This would be true even though as mentioned the 2-inch mesh gill net sample as a whole differs from the tagged population, it being only reasonable to suppose that although the nets later were more efficient at catching larger fish, they would still be equally effective at catching tagged and untagged fish of a given size. The test in Table 7 indicates that the two distributions do not agree. The ratios of tagged to untagged fish probably differ in different size groups of the population.

Table 7. Test to find whether walleyes of different lengths were tagged in proportion to their abundance, in 2-inch mesh gill net sample, 1953

	Length at last annulus			
	12-17.9	18-19.9	20-29.9	Total
Number untagged	136	39	40	215
Number tagged	9	10	8	27
Total	145	49	48	242
Expected proportion	.5991	.2024	.1983	

Chi-square = 9.29; highly significant, 2 degrees of freedom

Specifically it is implied that the size groups as found on the spawning grounds are not in proportion to their abundance in the population, and that they differ by including too few fish under 18 inches in total length. This might be expected since it is the smaller fish which would be less likely to be mature and would therefore not appear on the spawning grounds. In this connection, it might be profitable to compare the ratios of tagged to untagged walleyes in the three groups, these being 0.07 in the group from 12 to 17.9 inches in total length, 0.26 in the group from 18 to 19.9 inches, and 0.20 in the largest group. It has been reported that large female walleyes do not spawn each year (Deason, 1933; Carlander, 1945) which may account for the decline in the ratio in the largest size group. These ratios should be the same if the same proportion of each size group were on the spawning grounds. If the fish 18 to 19.9 inches in length are regarded as a standard, 25.6 per cent of the 136 smaller fish should have been tagged. Since only 9 were tagged, the implication might be made that only 26 per cent as many of the walleyes 12 to 17.9 inches in total length were mature.

In considering the experimental gill net sample, an examination of Table 5 reveals, without the necessity of a statistical test, that the frequency of size groups in this sample differs from that of the known tagged population. Even if only walleyes over 14 inches in total length are considered in the experimental gill net sample, the frequency distributions still differ. The ratio of tagged to untagged differs with the size of the fish (Table 8). It is interesting, however, that if the fish over 18 inches in length are considered as one group, which would be realistic in view of the small numbers involved, the ratio of tagged to untagged is 0.2, which is true also for the group from 16 to 18 inches in total length. The evidence from both the 2 inch and experimental gill net samples is therefore that fish over 16 inches in length probably were tagged in proportion to their abundance, since the ratios for the 2-inch mesh are about the same for these larger size groups as in the experimental gill net.

Table 8. Comparison of the numbers of tagged and untagged walleyes in size groups as found in the experimental gill net sample, 1953

	Total length in inches							
	6- 11.9	12- 13.9	14- 15.9	16- 17.9	18- 19.9	20- 21.9	22- 23.9	24 26.9
Untagged	43	52	84	30	10	1	3	1
Tagged	0	1	2	6	3	0	0	0

In view of the preceding discussion, it would seem desirable to derive the population estimate from the combined samples separated by size groups. The result at any rate would be to put the errors where they belong, in the smaller size groups of fish where a lesser percentage of them are tagged.

To make such a combined estimate it is still necessary that the two samples refer to the same population of fish. One piece of evidence in this connection is the ratio of tagged to untagged in the particular size

Table 9. Comparison of the percentages of tagged walleyes in size groups of experimental and 2 inch mesh gill net samples, 1953

Size groups by total length	Experimental gill net sample		2 inch mesh gill net sample	
	Number	% tagged	Number	% tagged
12-13.9	53	1.88	6	0
14-15.9	86	2.32	62	3.22
16-17.9	36	16.66	77	10.38
18-19.9	13	23.07	49	20.40
20-21.9	1	0	21	33.33
22-23.9	3	0	17	0
24-25.9	1	0	8	12.5
26-27.9	0	-	1	0
28-29.9	0	-	1	0

group for each sample. The ratio should be the same if the samples do indeed refer to the same population. In the 3 groups for which there is a comparison, the ratios do not seem greatly discrepant (Table 9).

It would be desirable to use a chi-square test on each of the size groups to determine whether a difference might probably exist. Unfortunately the small number of recaptures made leaves only one size group adaptable to this test, since expected values of less than 5 are subject to bias. Table 10 compares the percentage tagged in the 16-17.9 inch size group of the two samples. No difference is indicated.

Although a chi-square test is not appropriate for the other two size groups, it may be seen that the percentages tagged in the two samples are in closer agreement than for the 16-17.9 inch size group. It would seem reasonable to conclude, therefore, that no difference exists in the percentages of tagged fish by size groups in the two samples.

Table 10. Chi-square test to find whether any difference exists in the percentages tagged in the fish 16-17.9 inches in total length from two samples, 1953

	Tagged	Untagged	Total
2 inch mesh	8	69	77
Experimental mesh	6	30	36
Total	14	99	113
Expected proportion	.1238	.8761	

Chi-square = 0.89; non significant, 1 degree of freedom

Table 11. Estimates of the number of walleyes in each size group by combining two samples, 1953

Size group	Total tagged	Tagged in samples	Total samples	Estimate	95% confidence interval	% tagged
12-13.9	15	1	59	888	153 - ∞	1.69
14-15.9	581	3	148	28,762	8,803 - 193,666	2.02
16-17.9	444	14	113	3,586	2,254 - 7,161	12.38
18-19.9	295	13	62	1,407	799 - 2,169	20.96
20-21.9	63	7	22	198	113 - 450	31.81
22-23.9	13	0	20	260[a]	-	-
24-25.9	8	1	9	72	18 - ∞	11.11
26-27.9	2	0	1	-	-	-
28-29.9	1	0	1	-	-	-
	1,422	39	-	35,173	-	-

[a]The minimum estimate of 260 walleyes 22-23.9 inches in length was derived by substituting 1 tagged fish.

Randomness of tag returns

It has been demonstrated that good mixing of the tagged walleyes took place on the spawning grounds. Reports of tags from anglers during the fishing season demonstrate a spreading of the walleyes throughout the lake, with a few concentrations of tag returns from spots where fishermen tend to congregate (Fig. 1).

Estimate by size groups

The preceding sections suggest that perhaps the best available estimate of the number of walleyes in Clear Lake can be made by considering each size group separately as in Table 11. The confidence intervals are quite narrow for the walleyes from 16 to 22 inches in length. These may be considered the bulk of the spawning population. The widest limits are in size groups where most of the fish were immature. It is this group that creates the wide confidence limits in the estimates undivided by size groups. This is true because they are apparently the most abundant fish in the population, though not represented on the spawning grounds according to their abundance.

The percentages of fish tagged in each size group as seen in Table 11 shed further light on the rate of maturation of walleyes. A steady increase is noted except for fish 24 to 25.9 inches in length, and the point for the larger fish is based on a very small sample. A graph of these points (Fig. 2) shows that the curve of increase in availability on the spawning grounds (probably related to the increased percentage of mature fish with increase in length) may be represented by a straight line for fish over 14 inches in length with an increase of about 10 per cent in the percentage tagged for each 2-inch increase in length.

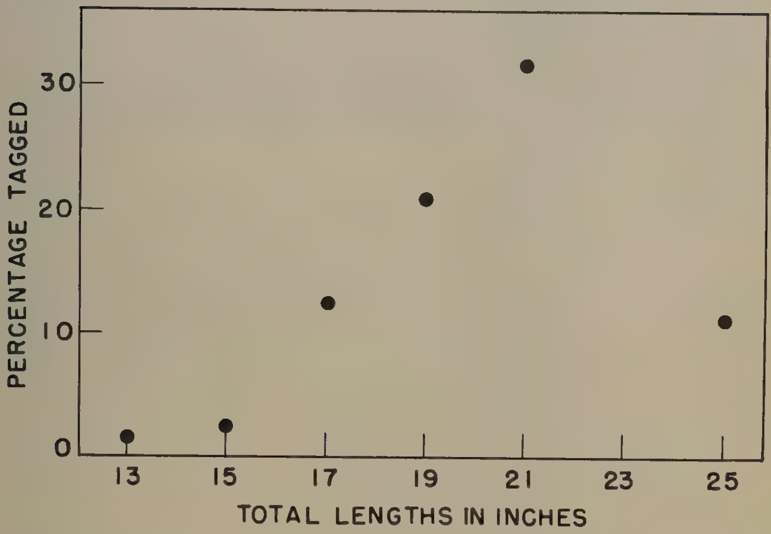

Fig. 2. Percentage of tagged walleyes at various lengths on spawning grounds, 1952 and 1953, reflecting an increase in availability with increased size

Deason (1933) and Carlander (1945) reported that large female walleyes may be sterile or miss spawning seasons. The decline in the availability of walleyes over 22 inches in length on the spawning grounds may be evidence of a decrease in the percentage spawning. The walleyes over 22 inches are probably mostly females (Table 1).

Estimate by age groups

Of interest in connection with the population estimate in size groups is an estimate of the number of walleyes of each age in the population. There is more than one approach to this problem. Cochran (1953, p. 90) discusses such a problem for a stratified sample and suggests that the proportion in each stratum and its variance be determined. In the present case, however, it seems desirable to make a somewhat different approach. Since scales were taken from a sample of fish during tagging, it is possible to estimate the total number of each age tagged. This may be done by strata as Cochran suggests. Stratification will reduce error in the present case, since a selection of larger fish was exercised as it was known their age is determined with greater difficulty than smaller fish. A sample of scales from 150 walleyes was taken during the tagging. Table 12 shows the number of walleyes of each age in each length grouping of the sample.

Since the number of walleyes tagged in each size group is known, it is possible to estimate from Table 12 the number of each age tagged

Table 12. Number of walleyes of each age in each length stratum in the sample taken during tagging

Total length in inches	Age								Total
	III	IV	V	VI	VII	VIII	IX	X	
14-15.9	24	3							27
16-17.9	14	23	4						41
18-19.9	1	17	27	3					48
20-21.9			14	8	1				23
22-23.9				1	4				5
24-25.9				1	3				4
26-27.9								1	1
28-29.9							1		1
Total	39	43	45	13	8	0	1	1	150

Table 13. Estimated number tagged of each age group, 1953

Size group	Age								Total
	III	IV	V	VI	VII	VIII	IX	X	
12-13.9[a]									15
14-15.9	516	65							581
16-17.9	152	249	43						444
18-19.9		104	166	18	7				295
20-21.9			38	22	3				63
22-23.9				3	10				13
24-25.9				2	6				8
26-27.9								2	2
28-29.9							1		1
Total	668	418	247	45	26	0	1	2	

[a]None scale sampled.

(Table 13). For example, in the stratum 14-15.9 inches in total length, 24/27 or 88.9 per cent of the 581 tagged are probably 3 years old, and 11.1 per cent of them 4 years old.

The number of fish of each age in the samples was then determined from scale samples. The population estimates in Table 14 follow from the data just discussed. Since the estimates obviously do not differ, they are combined as in part C of Table 14. Though the confidence intervals are not shown, they must be quite broad since two variables are present in the estimation, the number tagged of each age being itself only an

Table 14. Estimated number of walleyes of each age, 1953 samples

Age group	Number of fish in sample	Number tagged in sample	Number tagged of each age	Estimated population
		A. Two inch mesh sample		
III	78	3	668	17,396
IV	92	15	418	2,718
V	32	7[a]	247	1,129
VI	17	1	45	765
VII	14	1	26	359
VIII			0	
IX			1	
X			2	
				22,367
		B. Experimental gill net sample		
III	135	6	668	15,045
IV	33	5	418	2,759
V	6	0	247	
VI	5	1	45	225
VII	1	0	26	
VIII			0	
IX			1	
X			2	
				18,029
		C. Samples combined		
III	213	9	668	15,809
IV	125	20	418	2.612
V	38	7	247	1,341
VI	22	2	45	495
VII	15	1	26	390
VIII			0	
IX			1	
X			2	
				20,647

[a]Unfortunately scales were not readable on 1 tagged fish, but its length of 20.1 inches and the fact that it was a female make it most probably 5 years old.

estimate. Unfortunately the sample used for the estimation of the number of each age tagged did not include any walleyes less than 14 inches total length. For this reason the sum of the estimates by age may be expected to be less than that by size strata.

ERRORS IN THE ESTIMATES DUE TO LOSS OF TAGS

Another factor in addition to sampling errors which may influence the population estimates is loss of tags. During the 1953 tagging, 27 fish were noticed which had the premaxilla torn or damaged in such a way as to indicate that a tag might have been lost. A total of 40 fish tagged in 1952 were recaptured during the tagging in 1953. Assuming the 27 fish which seemed to have lost tags were all tagged in 1952, a loss of 40.29 per cent of the tags is indicated. A 95 per cent confidence interval places the loss at between 27 and 55 per cent. It may not be safe to assume either that all such damaged premaxilla represent lost tags or that if this is true that all of them were from fish tagged in 1952 and not in 1953. It would seem somewhat improbable, however, that by accident a fish should be injured in precisely the spot where tags were applied. It further seems likely that loss of tags during the short period of tagging would be slight.

Using the summer netting sample, it is possible to derive another estimate. Recaptures of 5 fish tagged in 1952 were made in the 2-inch mesh gill net. In addition 5 fish with damaged premaxillae were captured. If it is true that all such damaged premaxillae represent lost tags, a loss of 50 per cent is indicated, with a 95 per cent confidence interval of 19 to 81 per cent.

Another estimate, which does not depend on the assumption that the damaged premaxillae necessarily represent lost tags, may be derived using the data on growth which will be discussed in another paper. Unfortunately, the method suffers from other crudities. It depends on the fact that in 1952 an estimated 12.7 per cent of walleyes over 16 inches in total length were tagged (see Tagging and Results in 1952, p.58). The average annual increment of fish 16 inches long is found to be 2.3 inches. Roughly then, this same group of fish should be 18 inches or more in total length in 1953. A total of 115 walleyes were caught which were in this size range, 6 of them being tagged. Based on the idea that 12.7 per cent or about 15 of them should have been tagged, a loss of 9 tags or about 60 per cent is suggested. This estimate, though based on more fish and a greater number of tags is subject to considerable error, particularly since the estimate of 12.7 per cent is subject to normal error in addition to the number of recaptures in the sample of 115, and the estimate of growth.

At any rate all three approaches agree in suggesting that a large number of tags were lost. It is not clear by what degree the population estimates in 1953 should be corrected for loss of tags. The loss of 40 per cent seems to be the most reliable estimate, but this took place over a period of one year, while the sampling was done within 4 months of tagging. Assuming a constant rate of loss, the 40 per cent would amount fo 7.98 per cent loss per month. This would cause an upward

Table 15. Population estimates with number of recaptures corrected for loss of tags

	Total tagged	Tagged in sample	Corrected No. tagged	Total sample	Estimate
		A. By size groups			
Size group					
12-13.9	15	1	1.2208	59	728
14-15.9	581	3	3.3862	148	25,482
16-17.9	444	14	15.9806	113	3,140
18-19.9	295	13	14.8390	62	1,233
20-21.9	63	7	7.8522	22	177
22-23.9	13	0	-	20	-
24-25.9	8	1	1.1532	9	62
26-27.9	2	0	-	1	-
28-29.9	1	0	-	1	-
				Sum	30,822
		B. By age groups			
Age group					
III	668	9	10.3599	213	13,734
IV	418	20	23.6214	125	2,212
V	247	7	7.7061	38	1,218
VI	45	2	2.3076	22	429
VII	26	1	1.1538	15	338
VIII	0	-	-	-	-
IX	1	-	-	-	-
X	2	-	-	-	-
				Sum	17,931

distortion of the population estimate, since fewer recaptures would be made than should have been. Assuming the rate of loss to be the same for fish tagged in 1953 as in 1952, recaptures made in June should be increased by 7.98 per cent since a loss of this magnitude probably occurred in May. Similarly, July recaptures should be increased by 15.32 per cent and August recaptures by 22.08 per cent. Table 15 shows the population estimates by strata, corrected for loss of tags. A comparison with Table 11 shows that though loss of tags was high, the effect on the population estimates was only slight, because sampling was carried out over such a short interval of time.

ESTIMATES OF MORTALITY

If population estimates by ages can be made for two or more successive years, then at least some idea may be had of the extent of natural mortality, though the estimate will of course be affected by the reliability of the population estimates. Although population estimates have been made for two successive years for the walleyes in Clear Lake, it was not possible, due to the small number of recaptures, to subdivide the estimate of 1952 into estimates by age. This direct approach was therefore impossible in estimating the natural mortality of the walleye in Clear Lake.

It is possible, however, to subdivide the 1952 population estimate into an estimate by ages using the assumption that experimental gill nets catch the ages concerned in proportion to their abundance. The age class composition of the 1953 experimental gill net samples was quite similar to that estimated for the population (Table 14). A chi-square test gives a value of 6.45 which is not significant at the 95 per cent level with 3 degrees of freedom. If the 1952 experimental gill nets also caught walleyes in age groups III and older in approximate proportion to their abundance, the total estimate for 1952 could be subdivided on the basis of the frequency of occurrence in the experimental gill net catch for that year.

Evidence as to whether the experimental gill net caught walleyes in proportion to their abundance by ages in 1952 is meager. One approach, however, is to compare as in Table 16 the frequency of occurrence of 3, 4, 5, and 6 year olds in 1952 with the frequency of occurrences of 4, 5, 6, and 7 year olds in the 1953 experimental gill net catch. No difference is indicated.

Since the best 1952 estimate was 6030 walleyes over 16 inches, only the 89 walleyes over 16 inches long taken in the experimental gill nets were utilized in estimating the numbers in the various age groups.

Table 16. Comparison of the frequency of occurrence of certain year classes of walleyes in experimental gill net catch of two successive years

	Year class			
	1949	1948	1947 and 1946	Total
No. of walleyes in experimental gill nets in 1952	69	28	22	119
In 1953	33	6	6	45
Total	102	34	28	164
Expected proportion	.622	.207	.171	1.000

Chi-square = 3.4; non significant, 2 degrees of freedom

Furthermore, only the age groups from IV on could be used because most of the younger fish were under 16 inches total length. From the 1952 and 1953 estimates for the 1946-8 classes the annual mortality was estimated at about 35 per cent (Table 17).

Table 17. Annual mortality as estimated from the numbers of walleyes in the 1946-48 year classes in 1952 and 1953

Year class	Estimated number in 1952	1953	Mortality (%)
1948	1896	1341	29.0
1947	677	495	26.9
1946	814	359	55.9
Total	3387	2195	35.2

In 1952, fishermen returned 126 tags representing 15.7 per cent of all walleyes tagged. Natural mortality may therefore be estimated by subtracting 15.7 per cent from the total mortality.

Another approach to the estimation of natural mortality may be derived from the fact that fishermen returned 91 or 6.3 per cent of the tags from walleyes tagged in 1953. If we are willing to assume they also caught 6.3 per cent of the walleyes tagged in 1952 which remained, an estimate of the total number of 1952 tags in the population can be made. Fishermen returned 9 tags in 1953 from walleyes tagged in 1952. If these represent 6.3 per cent of the total number, an estimate of 143 walleyes tagged in 1952 and remaining in 1953 is made. Of the original 801 tags, 126 were returned by fishermen in 1952 leaving 675 tagged, an estimated 40 per cent or 320 were shed or lost during the year leaving 355 tagged, and the 143 estimated still remaining in 1953 leaves 212 unaccounted for. These 212 probably are best assigned to natural mortality, giving an estimate of 27.4 per cent.

Still another approach uses the fact that 6 walleyes tagged in 1952 were recaptured during the netting operations in 1953. The ratio of 1952 tags to 1953 tags in the samples, or 6/39, is an estimate of the ratio of the total populations of tagged fish. The total number of 1431 walleyes tagged in 1953 is taken as the constant to give an estimate of 220 walleyes tagged in 1952 still tagged and alive in 1953. A loss of 135 walleyes or 16.8 per cent is indicated up to the beginning of summer, 1953.

SEX RATIOS AND MOVEMENTS OFF SPAWNING GROUNDS

It would appear that the sex ratio is quite variable on the spawning grounds (Table 18). Eschmeyer (1950) has reviewed the ratios as found by various authors. In the seven studies noted, including his own, the ratio varied from 2:1 to 15:1 in favor of the males. It would appear that the sex ratio is not constant even in the same body of water. In Clear Lake, for instance, in 1952, of the 793 walleyes which were sexed during tagging, 281 or 35.4 per cent were females. This indicates a ratio of

Table 18. Water temperatures and number of walleyes in the catch on certain nights of the 1953 spawning run

Date	Air Temp. °F	Water Temp. °F	Catch	Number of females	Sex ratio
April 9	--	41	24	9	6:1
10	32	40	56	5	10:1
11	30	38	146	7	20:1
12	28	38	94	15	5:1
13	36	43	78	3	25:1
14	--	44	58	12	4:1
17	26	40	26	0	--
18	29	40	35	13	2:1
19	30	40	77	9	--
20	35	42	91	4	8:1
21	50	46	19	7	2:1
22	--	48	28	4	6:1
25	42	45	38	9	3:1
26	31	44	19	0	--
27	40	44	46	2	22:1
28	39	45	8	4	1:1
30	49	45	21	0	--
May 1	49	--	12	0	--
4	49	47	23	7	2:1
5	51	51	3	-	--
6	57	52	2	-	--
7	--	60	2	-	--
13	--	54	19	-	--
19	63	60	2	-	--

about 2:1. In 1953, however, only 142 out of 1,431 walleyes tagged were females. This is a ratio of about 9:1 in favor of the males. The water stayed cold rather late in 1953 so that maturation of many females could have been delayed. The prolonged spawning season may have permitted a greater sampling of males which apparently remain on the spawning areas while females come in only when ripe. After May 4, 1953, few walleyes were taken which could be sexed. On this date 10 out of the 23 could be sexed. On May 13, 1953, only 4 out of 19 could be identified as males.

Although dissections were not made to determine the sex ratio after spawning when the walleyes had moved to other areas, there is another method of arriving at this estimate. It was possible to estimate the total population of males on the spawning grounds as 12,119. Later in the summer it was possible to estimate the total combined population as

30,822. The difference, 18,703, includes immature males and both mature and immature females. It would therefore appear that the real sex ratio as found in the population at large does not differ greatly from a 1:1 ratio.

The reason for the difference on the spawning grounds is probably that the males arrive sooner and stay longer. Eschmeyer (1950) found this to be true as did Eddy and Surber (1947). Furthermore, males probably mature at an earlier age (Carlander, 1945; Deason, 1933; Hile, 1954). There is also evidence that female walleyes tend to be longer lived than males (Carlander, 1945; Hile, 1954).

The gradual decline of catch on the spawning grounds evident in Table 18 indicates a movement off the grounds. In 1952, sport fishing for the walleye was apparently most successful on rock reefs near the spawning grounds. The greatest number of tag returns were from the early part of the season and from the rock reefs. As the returns tapered off, they also shifted location from the rock reefs on the south shore to vegetated areas on the north shore.

Fishing success was uniformly poor in 1953 so that no such picture developed. Netting was carried on almost constantly, however, in an attempt to locate the walleyes. The greatest success finally occurred at night in June and July in areas of vegetation along the north shore.

It is possible that movements into the areas of vegetation occur in search of food and perhaps cover. At sunrise on July 13 and again on July 15, 1953, a number of walleyes were taken from nets which had been set during the night. On being released, these fish merely swam to the bottom in about 2 feet of water and groped around until their heads were covered by algae.

The negative phototropism of the walleye is well known and some authors (see Eschmeyer, 1950 for a review) have pointed out that the walleye moves to deeper water to avoid the light. In Clear Lake, 35 per cent of the surface area is less than 10 feet deep and only 15 per cent is over 15 feet deep (Bailey and Harrison, 1945). Pearcy (1952) reported Secchi disc readings averaged 7.3 feet in June and 5.3 feet in July, 1951. It would appear that these areas of vegetation provide shade enough in water 7 to 10 feet deep to satisfy the walleye. At night, movements occur into shallower water (see Carlander and Cleary, 1949).

SUMMARY OF THE ESTIMATES

The various population estimates which have been made are summarized in Table 19. Of the 1952 estimates, the estimate of 6,030 walleyes over 16 inches in total length is probably the most reliable and meaningful. Causes of distortion of the other estimates have been discussed. The estimate of 12,665 spawning walleyes is probably not seriously in error, but it is difficult to relate the spawning population to the total population in 1952.

The 1953 estimates are, in general, more reliable because of the greater number of recaptures. The estimate of 16,311 walleyes on the spawning grounds is distorted by emigration of females. The estimate referring only to males is probably quite accurate. The estimates made

Table 19. Comparison of the various population estimates

	Total Population
1952	
During tagging	12,776
Creel census	11,831
Experimental gill net uncorrected for size	61,615
Experimental gill net-walleyes over 16 inches total length	6,030
1953	
During tagging	16,311
Males only	12,119
Creel census	17,179
Experimental gill net uncorrected	28,169
2 inch mesh gill net uncorrected	12,985
Combined camples by size groups over 12 inches	35,173
Combined samples by size groups over 12 inches in total length corrected for loss of tags	30,822
Combined samples-walleyes over 16 inches total length corrected for loss of tags	4,872
2 inch mesh sample by ages, walleyes 3 and older	22,367
Experimental mesh by ages, walleyes 3 and older	18,029
Combined samples by ages, walleyes 3 and older corrected for loss of tags	17,931

from samples uncorrected for differences in size from the tagged population suffer from inaccuracies, but the estimate from the combined samples by size groups over 12 inches in total length and corrected for loss of tags is certainly the most accurate estimate available for 1953. This estimate, 30,822, corresponds to 8.5 walleyes over 12 inches in total length per acre of surface on the lake. Rose (1955) estimated the adult population of walleyes (over 13 inches) in Spirit Lake, Iowa, as about 7 per acre in 1954 and about 5 per acre in 1947, and in Storm Lake, Iowa, as 21 per acre in 1948 (Rose, 1949).

The estimate by ages does not include 1- and 2-year-old fish so that it is certainly an underestimate and is of less utility than the estimate by size groups since the latter includes some of these fish. The estimate by ages is also subject to errors due to misread scales.

The only estimates which are directly comparable for the two years are the estimates of the number of walleyes over 16 inches in total length. Probably the total populations did not differ greatly in the two years studied. If anything the 1953 population might be somewhat smaller than the 1952 population. It would appear, however, that an increased number of males were present on the spawning grounds in 1953. This increase could be due to the maturation of males from the 1950 class, which, according to scale studies, was an abundant year class.

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A NONVISUAL METHOD FOR TRANSPORT NUMBERS IN PURE FUSED SALTS. II*

Frederick R. Duke and James P. Cook

Institute for Atomic Research and Department of Chemistry
Iowa State College, Ames

ABSTRACT

An extension of a modified Hittorf method for the measurement of transport numbers of fused salts is described and the results with sodium and potassium chlorides are recorded. Although the precision is not great, values of 0.77 and 0.87 are obtained for the transport number of the positive ions in KCl and NaCl, respectively.

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In a previous paper (1) the authors reported a determination of the transport number of silver nitrate by following the changes in distribution of the salt which take place when a direct current is passed through a cell of unglazed porcelain containing the fused salt. In the present paper the work is extended to sodium and potassium chlorides.

EXPERIMENTAL

In the preliminary experiments in this work it was attempted to employ a porous porcelain cell such as was used in the previous work on silver nitrate. The cell was held in slots cut in the ends of two five-eighths inch pieces of spectroscopic graphite and the entire apparatus was kept in a chlorine atmosphere. This approach was not successful because of excessive vaporization of salt from the surface of the unglazed porcelain. Accordingly, the procedure was modified in the following manner: The salt was dry mixed with diatomaceous earth which had been washed with nitric acid and dried. The proportion of salt to earth ranged from one to five per cent salt by weight. Smaller amounts of salt resulted in analytical difficulties and larger amounts tended to flow under the influence of gravity.

The mixing of the dry salt and diatomaceous earth was carried out in the following manner: The pulverized dry salt, together with the desired amount of diatomaceous earth, was introduced into a mixing apparatus consisting of two 57-mm pyrex tubes joined in the center to make a 60° angle. The open ends were closed with polyethylene-covered rubber

*Contribution No. 636. Work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission.

Table 1.

		Total Equivalent	Change	Current in Faradays	T^+
NaCl					
	temp. 850°C				
1		.009028	.000109	.0001404	0.77
2		.006503	.001203	.001035	1.16
3		.006770	.000662	.000806	0.82
4		.004434	.000361	.000405	0.89
5		.005824	.000433	.000595	0.73
	using Al_2O_3				
6		.010834	.000314	.000343	0.91
7		.009073	.000443	.000525	0.84
				Average 0.87 ± .06 std. dev.	
KCl					
	850°C				
8		.004632	.000304	.000453	0.67
9		.012469	.000363	.000550	0.66
10		.012901	.000346	.000481	0.71
11		.002819	.000315	.0003918	0.80
12		.01290	.00083	.00098	0.84
				Average 0.77 ± .08	

stoppers and the apparatus rotated about its long axis about 500 times with such shaking as seemed expedient until a uniform mixture was achieved. The mixture was then packed firmly and uniformly in a vycor cell approximately 0.68 cm in inside diameter and 2.10 cm in length, with one end constricted to receive a platinum wire or 0.32-cm spectroscopic graphite rod to serve as anode. In certain runs a solid silver anode was also used. The cathode was a 0.67-cm graphite rod butted firmly against the diatomaceous earth cell with provision for the introduction of dry chlorine. The cell was placed in an electric tube furnace and heated to the desired temperature.

The current source was a 45-volt dry cell with a milliammeter and iodine coulometer in series to measure the current and check the progress of the run. The total current strength varied between runs from about three to ten milliamperes depending on the concentration of the salt and the temperature.

At the conclusion of the electrolysis the cell was removed from the horizontal electric tube furnace and allowed to cool. The cell was then

divided into an anode and cathode compartment and analyzed for total chloride by gravimetric chloride or ion exchange conversion of the salt to hydrochloric acnd and titration with carbonate-free standard sodium hydroxide. The original salt concentration was determined by the total salt found at the conclusion of a run. Blank experiments indicated that distribution of the salt was uniform in the absence of an electric current.

The transport number was calculated from the following relationship:

$$\frac{\text{Change of equivalents in anode due to electrolysis}}{\text{Faradays of electricity passed}} = T^{+}.$$

In order to check the effect of different dispersing media, two runs were made using aluminum oxide instead of diatomaceous earth. The results with sodium chloride were independent of the nature of the dispersing medium.

The results of several runs on sodium chloride and potassium chloride are given in Table 1.

DISCUSSION

While this method does not appear capable of as good precision as the bubble cell or radio tracer methods (2, 3) certain conclusions can be drawn.

Sodium and potassium chlorides seem to be chiefly cation conductors, in agreement with the work of Mulcahy and Heymann (4). Since a larger fraction of the current is carried by the sodium ion in sodium chloride than the potassium ion in potassium chloride, it is suggested that the size of the ion in these salts is a factor in the migration of the ions.

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INDUCED ESTRUS IN OVARIECTOMIZED COWS[1]

R.M. Melampy and J.M. Rakes

Department of Animal Husbandry
Iowa Agricultural and Home Economics Experiment Station
Iowa State College, Ames

The control of estrus and ovulation in farm animals is desirable from the standpoint of the animal breeder as well as the livestock producer. However, it is necessary to study the action of various hormones associated with reproductive processes before practical use can be made of them in the control of reproduction.

According to Asdell et al. (1945) the average level of estradiol benzoate required to induce estrus in ovariectomized heifers is 600 R.U.[2] daily for three days. Melampy et al. (1957) have found that progesterone can induce estrous behavior, including sexual receptivity, in the estrogen-conditioned ovariectomized cow. Similar results have been reported for other species. The synergistic action of progesterone with estrogen has been reported by Dempsey et al. (1936) for the guinea pig, by Boling and Blandau (1939) for the rat, by Ring (1944) for the mouse, and by Frank and Fraps (1945) for the golden hamster.

The investigation reported here deals with the analysis of the estrous response of ovariectomized cows receiving different levels of estradiol benzoate in combination with progesterone.

EXPERIMENTAL PROCEDURE

Four breeds of dairy cattle, including Holstein, Ayrshire, Guernsey, and Jersey were used to furnish animals in the three experimental groups employed in this study. The number, age, and weight of each cow are indicated with each group as follows:

Group 1
H-3955, 2 yrs., 1000 lbs.
A-3867, 3 yrs., 1000 lbs.
H-3991, 2 yrs., 1000 lbs.

Group 2
H-3991, 3 yrs., 1200 lbs.
J-3560, 4 yrs., 900 lbs.
G-3969, 4 yrs., 900 lbs.
H-4025, 2 yrs., 1000 lbs.

Group 3
H-3991, 3 yrs., 1200 lbs.
G-3969, 4 yrs., 900 lbs.
H-4025, 2 yrs., 1000 lbs.

[1]Journal Paper No. J-3441 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1325.

[2]R.U. = Rat Units. One mg of crystalline estradiol benzoate has a potency of 6,000 Allen-Doisy Rat Units.

Cow H-3991 was used in Group 1 and approximately one year later was used again in Group 2 and in Group 3. Cows G-3969 and H-4025 were both used in Groups 2 and 3. All cows were checked for the occurrence of normal estrous cycles prior to bilateral ovariectomy. The experimental animals were maintained in a small lot and were fed ad libitum alfalfa hay and oat straw. Several bulls were available in an adjoining area for checking individually the estrous response of each cow.

The animals in Group 1 were on experiment for a period of 15 days consisting of three 5-day intervals. During the first 5-day interval they received a daily subcutaneous injection of 0.4 mg of estradiol benzoate in sesame oil, during the second 0.8 mg, and during the third 1.6 mg per day. The cows in Group 2 were on experiment a total of 11 days and were daily injected subcutaneously with 20 mcg of estradiol benzoate per 100 lbs of body weight and 1 mg of progesterone. This dosage of estrogen is subminimal in terms of the level required to induce estrus (Melampy et al., 1957). Animals in Group 3 were given a single injection of 3 mg of estradiol benzoate which was ten times the minimal level of hormone required to induce full estrus in these cows. It is of interest that a minimal dosage of 0.3 mg of diethylstilbestrol also induced bull acceptance in these cows.

In the case of Groups 1 and 2 observations were made for the duration of the experiments at three-hour intervals on the degree of estrus, vaginal temperature, mucus production, and reaction of the vulva. The degree of estrous behavior was scored by the following system: 0, cow shows no signs of estrus; +, cow is restless and followed by other cows; ++, cow mounts and stands for other cows; and +++, cow accepts bull. The vaginal temperature was taken by placing the bulb of the thermometer at a depth of approximately four inches. Mucus production and vulval reaction were evaluated by using 0 for no response to +++ for maximal activity. Only observations on estrous behavior were made on the cows in Group 3.

RESULTS

The data obtained from observations on the cows in Group 1 dealing with the degree of estrus, vaginal temperature, flow of mucus, and reaction of the vulva are summarized in Fig. 1. Fig. 2 presents similar data from the cows in Group 2. The results from animals in Group 3 are as follows: G-3969 showed +++ estrous behavior for a total of 15 hours whereas both H-3991 and H-4025 indicated bull acceptance for 27 hours.

DISCUSSION

As shown in Fig. 1 the incidence of +++ estrous behavior (bull acceptance) in each cow increased as the daily dosage of hormone was raised from 0.4 to 1.6 mg. During the first 5-day interval of observations H-3955 and A-3867 indicated a total of 12 and 21 hours of bull acceptance whereas H-3991 did not attain full estrous behavior. The total time of +++ behavior during the second 5-day interval was 9, 24, and 12 hours for cows H-3995, A-3867, and H-3991, respectively. These animals had

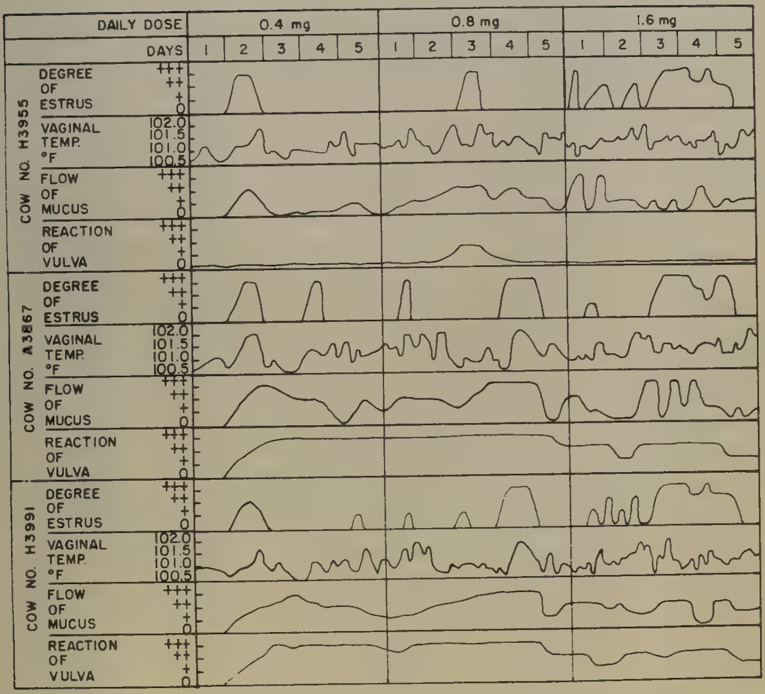

Fig. 1. Effect of different doses of estradiol benzoate on estrus in ovariectomized cows.

received 0.8 mg of estrogen daily. During the third 5-day period of this experiment the daily hormone dosage was 1.6 mg and the total hours of bull acceptance increased to 30, 27, and 21 for cows H-3955, A-3867, and H-3991 (Fig. 1).

The average vaginal temperature of the three estrogen-treated ovariectomized cows during the 15-day experimental period was 101.1°F. As shown in Fig. 1, there was no correlation between the vaginal temperature and the degree of estrus observed. Vollmann and Vollmann (1942) have reported a study of the basal temperature of the cow during the estrous cycle and they found that the lowest value (100.2°F) is associated with the time of ovulation. The highest value (101.7°F) occurs during the luteal phase of the cycle (day 14) and this is followed by a gradual decrease as the next follicular phase develops. Rubenstein (1938) observed a constant relationship between the lowest body temperature during the human menstrual cycle and the characteristic ovulation smear, and

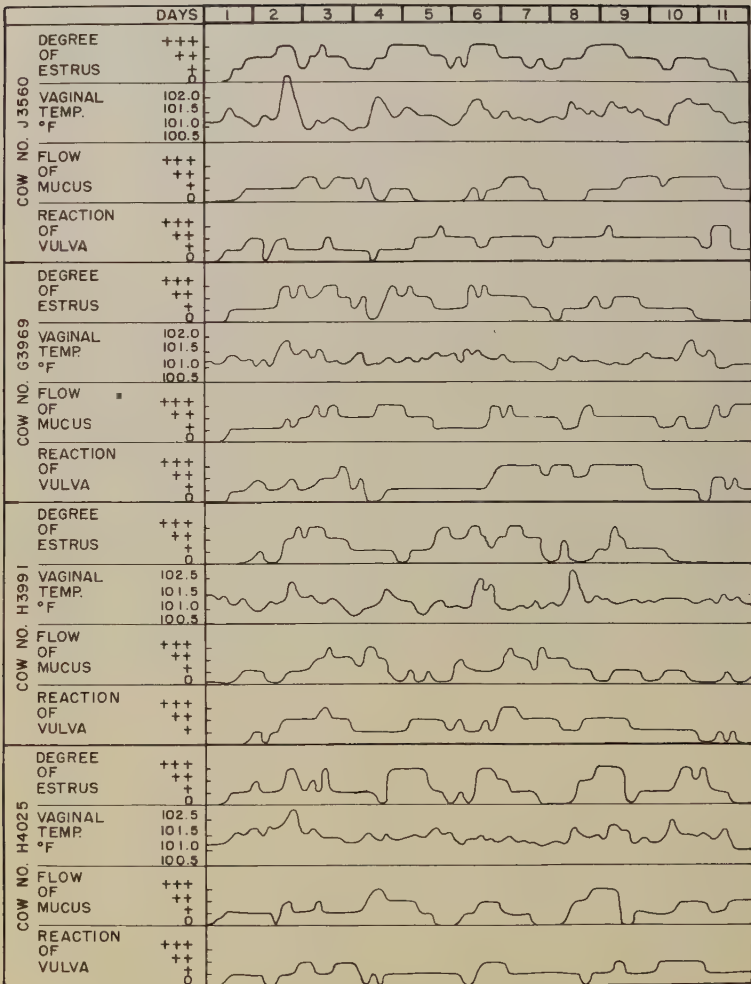

Fig. 2. Estrous response of ovariectomized cows receiving daily 20 mcg of estradiol benzoate per 100 lbs body weight and 1 mg of progesterone.

between the highest body temperature and the premenstrual smear. He concluded that the temperature cycle was as trustworthy an indicator of the ovarian cycle in the human female as the vaginal smear. According to Palmer (1949), follicle rupture is characterized by abrupt temperature rise in women during ovarian cycles in which conception occurred.

Mucous production was observed in all three cows following administration of estrogen. This response was more marked in A-3867 and H-3991. Mucus is discharged from the vagina of ovariectomized cows at levels of estrogen injection below 5000 Rat Units per day according to Asdell et al. (1945). However, in the experiments reported here, muc s production was observed in cows receiving as much as 9600 Rat Units (1.6 mg) daily.

Roark and Herman (1950) have reported that the secretion of mucus is the greatest during the first three hours of estrus in the cow. The response of the vulva was also greater in A-3867 and H-3991 than in H-3955. Furthermore, the udders of all three cows were stimulated by the third day of the second experimental period.

The cows in Group 2 which received daily 20 mcg of estradiol benzoate per 100 lbs body weight and 1 mg of progesterone showed the following hours of bull acceptance during the 11-day experimental period: J-3560, 75 hours; G-3969, 48 hours; H-3991, 54 hours; and H-4025, 81 hours. In a previous study Melampy et al. (1957) observed the duration of +++ estrous behavior ranged from 6 to 12 hours in cows receiving a single injection of a conditioning level of estrogen and 1 mg of progesterone.

The vaginal temperature of the four cows in Group 2 fluctuated during the experimental period as is shown in Fig. 2. In some instances the highest temperatures were associated with periods of bull acceptance whereas in others they were not. The average vaginal temperature of the four cows was 101.27°F which was 0.17°F higher than the average observed for Group 1. This small increase in vaginal temperature may have been due to the thermogenic action of progesterone. Forbes (1950) has observed that the injection of estrogen does not elevate waking temperature or basal temperature. However, the administration of progesterone is followed by a conspicuous temperature elevation in normal, post-menopausal, and castrate women and in men. As shown in Fig. 2 there was considerable variation in the flow of mucus as well as the response of the vulva throughout the experimental period. However, the reaction of the vulva tended to be greater during the second half of the experiment. Stimulation of the udders was observed on the eighth day of injection and was similar to that observed in the cows in Group 1.

In experiments reported by Asdell et al. (1945) the average level of estradiol benzoate required to bring ovariectomized heifers into heat was 600 R.U. daily for three days. The duration of heat was usually less than one day even though the injections were continued. In the normal cow this threshold is probably reached early in the development of the follicle. According to Asdell et al. (1945) estrous block, apparently in the central nervous system, then sets in, so that the cow is out of heat before ovulation takes place. However, estrous block was not observed in the cows either in Group 1 or Group 2 in the experiments reported here.

Bleeding was not observed in any of the cows during the period of estrogen administration or following hormone withdrawal.

SUMMARY

The induced estrous behavior response in ovariectomized cows became greater as the daily estrogen dosage was increased. Ovariectomized cows receiving 20 mcg of estradiol benzoate per 100 lbs body weight and 1 mg of progesterone showed intense estrous behavior. No relationship was observed between the estrous behavior response and the vaginal temperature in hormone-treated ovariectomized cows. The production of mucus and the reaction of the vulva were variable during the experimental periods. Estrous block was not observed in cows receiving daily injections of estrogen alone or in combination with progesterone. The average vaginal temperature of animals given estrogen and progesterone was higher than in animals receiving only estrogen. This observation suggests the thermogenic action of progesterone in the cow.

Bleeding was not observed in estrogen-treated ovariectomized cows.

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THE EROSION-LITTORAL ZONE OF NORTH TWIN LAKE, AND ITS RELATION TO DREDGING[1]

John B. Owen[2]

Department of Zoology and Entomology
Iowa State College, Ames

ABSTRACT

The erosion-littoral zone was found to be significantly wider in the southern end of North Twin Lake than at the northern end, probably because the dredged area at the southern end served as a settling basin for silt. Watershed and shore erosion result in covering some of the erosion-littoral sands with unproductive silts. Mayfly nymphs (Caenis sp.) and caddice fly larvae (Oecetis inconspicua) were the predominant bottom fauna organisms in the erosion-littoral zone. Beside being an important spawning and feeding area for game fishes, the erosion-littoral has important recreational values.

INTRODUCTION

In the drought years of the 1930's, many of the shallow prairie lakes of recreational value in Iowa gave evidence of imminent extinction. Former good fishing lakes became choked with emergent vegetation and became subject to annual winter kill. As an emergency remedy for certain of the state's critically shallow lakes, the Iowa State Conservation Commission initiated a program of dredging to restore the water depths.

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[2]Now Fishery Research Biologist, U.S. Fish and Wildlife Service, Juneau, Alaska. The author wishes to express his most sincere appreciation to Dr. Kenneth D. Carlander for guidance, personal assistance, and the highest measure of encouragement and support during the course of this investigation. Thanks are due Dr. Joseph Kutkuhn for assistance and cooperation in this study while he was working at North Twin Lake. Thanks are also due to Lee Tebo for assistance in the field and to C.E. Treman, publisher of the Rockwell City Advocate for the use of photographs in Figs. 2 and 3. Mr. Henry Pontius was very helpful during the field work at North Twin Lake. This opportunity is taken to express deepest gratitude for the many hours of assistance on the project given by my wife, Alice Koning Owen.

Fig. 1. North Twin Lake, May 30, 1939.
(U.S. Department of Agriculture photograph).

To date the policy of the Conservation Commission has been to consider dredging of Iowa's natural lakes as a last resort or emergency measure only. They have recognized that the long range ecological and limnological effects of such dredging operations are virtually unknown. They deferred adopting a general policy of dredging natural lakes until there was opportunity for the accumulation of scientific data and evaluation of dredging.

North Twin Lake (Fig. 1) in north central Calhoun County, was selected for this investigation of the ecological effects of dredging. Approximately 135 acres in the southern end of the lake were dredged to a depth of 14 to 18 feet in 1939 and 1940. The biological investigations were started in June, 1951. By this time it was expected that conditions in the undredged and dredged portions of the lake would be fairly stabilized.

Fig. 2. North Twin Lake and South Twin Lake in foreground.
(Photo by C.E. Treman, Rockwell City Advocate)

Fig. 3. North Twin Lake and South Twin Lake. Flood in spring of 1954.
(Photo by C.E. Treman, Rockwell City Advocate)

North Twin Lake is approximately two and one-half miles long and about one-quarter mile wide, with a surface area of 509 acres. The lake is situated in a level glacial plain which is devoid of striking features. North Twin Lake and neighboring South Twin Lake are glacial relicts. The lakes are situated in depressions in the ground moraine topography, left behind by the Mankato Lobe of the Fifth Wisconsin glaciation (Thwaites, 1935). The watershed of North Twin Lake is estimated to be 2,155 acres exclusive of the lake itself (Iowa State Planning Board, 1935). The watershed of the lake has been gradually enlarged by the installation of a network of drainage tiles, many of which drain into North Twin Lake. The various tile systems lead across shallow divides and drain former glacial potholes. In the spring of 1954, unusually heavy rains swamped the tile systems in the countryside about the Twin Lakes. The potholes were temporarily flooded, and aerial photos give a hint of the original pothole topography before artificial drainage (Figs. 2 and 3).

The lake has low earthen banks, from two to five feet high for the most part, although one or two knolls ten to thirty feet high form part of the northeastern shore. Traces of an old "wall" of glacial boulders may be seen along the low western shore of the lake.

The lake in the years 1951 to 1955 was from four to seven feet deep in the northern end, and from 9 to 14 feet deep in the dredge cut in the southern end. The lake bottom is mud except for a narrow zone of sand around its shoreline.

The survey of North Twin Lake by the Iowa State Planning Board in 1935 indicated that the original capacity of the lake basin was 72 per cent filled with silt. At that time, the average depth of the water was 5.85 feet, and large areas were grown up in higher aquatic plants. The recreational value was endangered, and it was decided to restore the depth of the lake by dredging out the basin. In 1939 and 1940, an area of 135 acres in the southern end of the lake was dredged to a depth of 14 to 18 feet (Fig. 4).

North Twin Lake is so shallow, even after dredging, that the entire lake bottom falls within the littoral zone as defined by Carpenter (1928). The littoral bottom of North Twin Lake presents the two typical phases described by Carpenter. The first, or "erosion-littoral" region, is a rather narrow sandy beach which extends around the entire lake. The second region, or "quiet-littoral" zone comprises the remainder of the lake floor.

The sandy erosion-littoral zone extends from the foot of the cut banks to the water's edge and out into the lake for a variable distance. The sand of the erosion-littoral zone is washed free from the glacial till and kept clean by the continued scouring of wave action.

The quiet-littoral region within the lake is at depths protected from wave action. The quiet-littoral lake bottom has become covered with accumulated silt and sediments. A survey in 1954 indicated that 92 per cent of the area of the lake bottom was covered with sediments, while only 8 per cent of the bottom area was to be found in the fringe of erosion-littoral sands.

The waters are highly eutrophic, reflecting the rich agricultural soil of the watershed and basin. Dissolved oxygen is well distributed from the surface waters down to near the bottom, attesting to effective circulation

and aeration of the water mass. Apparently the lake is so shallow and exposed to the wind that it does not stratify thermally for any significantly continuous length of time during the summer.

Turbidity of North Twin Lake water varies greatly. At times in some summers, the water supports a blue-green algal bloom. Temporary turbidity is also caused by storms which stir the bottom deposits. The shallow water is often noticeably stirred by heavy usage of motorboats on holidays and weekends.

Higher aquatic plants are virtually nonexistent in North Twin Lake proper. Some cattails, Typha spp., arrowheads, Sagitarria spp., and bullrushes, Scirpus spp., grow along the margins of Muddy Bay, however.

The lake has long been a valuable recreational site in that part of Iowa. It was reportedly a fabulous duck hunting area at about the turn of the century. A resort hotel was built on the lake shore at about that time, and hunting parties are said to have come to the Twin Lakes from as far away as Chicago.

As time went on, most of the swampy glacial depressions were artificially drained, and duck hunting about the Twin Lake has declined to a low ebb. However, the recreational value of this body of water has continued to grow, but in an entirely new form. North Twin Lake can now be termed a general aquatic recreational area. The lake is state-owned with a state park, swimming beach, picnic facilities, and numerous other state-owned access points for launching boats (Fig. 4). The lake provides fair to good fishing for black bullhead, Ictalurus melas (Rafinesque), yellow bass, Roccus mississippiensis (Jordan and Eigenmann), yellow perch, Perca flavescens (Mitchill), walleye, Stizostedion vitreum vitreum (Mitchill), northern pike, Esox lucius Linnaeus, black crappie, Pomoxis nigromaculatus (LeSueur), and largemouth bass, Micropterus salmoides salmoides (Lacepede).

In addition to the public facilities, there are between three and four hundred privately owned cabins around the lake. The cabins serve as summer residences or vacation spots for local residents, most of whom live within 25 miles of the lake. The cabins are along most of the eastern side of the lake and along the southwestern side. Many of the cabin owners have built retaining walls or have riprapped to stabilize their waterfront embankments.

MEASUREMENT OF EROSION-LITTORAL ZONE

The erosion-littoral zone in North Twin Lake was mapped August 10 and 11, 1954, at a time when the lake surface was at the level of the sill of the outlet structure. The survey was made by going around the entire lake shore and at suitable intervals sampling the bottom from the water's edge out into the lake along a graduated line perpendicular to the shore. The bottom was sampled by surface-diving, and the width of the erosion-littoral zone was taken to be the extent of the clean sandy bottom from the water's edge out to where the sand was replaced by silt or sediments.

Examination of the map of the sandy erosion-littoral zone suggested that the clean, wave-washed shore zone might be substantially wider in the southern end of the lake. It was felt that perhaps the dredge cut in

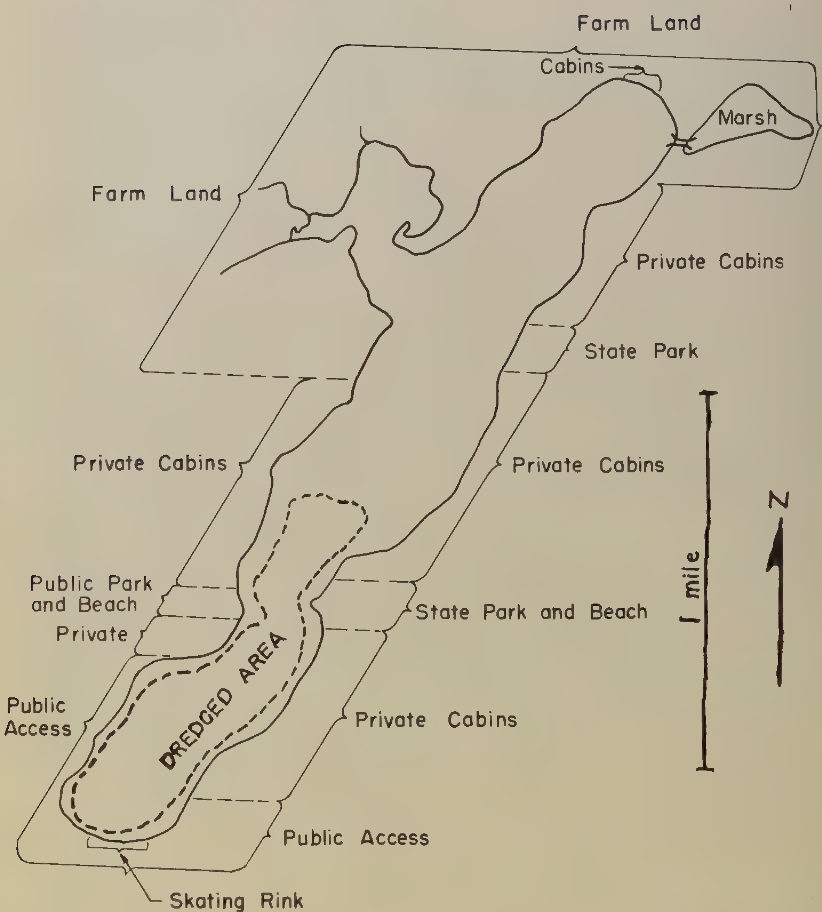

Fig. 4. North Twin Lake showing 135 acres dredged area and land use around the shore

southern end of the lake might be a factor in assisting in the maintenance of a wider erosion-littoral area in that end. The average width of the erosion-littoral zone was found to be 57 feet in the northern undredged end and 77 feet in the southern dredged end of the lake. The area of the erosion-littoral zone in the southern end was 9.33 acres per mile of shore line for a total of 24.0 acres for the 2.57 miles in that end. In the northern segment, there were 3.03 miles of shore line with 6.91 acres of sandy littoral zone per mile for a total of 20.9 acres.

SOURCES OF SILT AND SEDIMENTATION

During the survey of the shore line, some particular areas of what appeared to be escessive erosion were seen. There were three such areas of accelerated erosion, each of which seemed to be a source of undue amounts of silt entering the lake basin. The first area was a cultivated field embracing the northern end of the lake (Fig. 4). This field was in oats in 1954, and sheet erosion and minor gullying were much in evidence at that time (Fig. 5). At a time of low water earlier in the season, this field had been plowed and a seed bed prepared operating the farm equipment across the dry slough. The slough drains into North Twin Lake when there is sufficient runoff. At the time the photographs (Figs. 5 and 6) were taken, the unprotected soil of the field had been washed as far as the slough; at the next rain, much of the soil would go into the lake.

In the northern end of the lake, near the outlet of the culverts from the slough, there was direct evidence of periodic inflows of top soil. In places near shore, the former firm erosion-littoral sand could be felt through a layer of a few inches of soft black mud. In other places, about 8 inches of mud was overlain by a thin layer of sand. It appeared that a flood of mud came into the lake covering up the sandy erosion-littoral area; later, when the flow ceased, the situation stabilized, and fresh sand from the shore line gradually was spread over the new bottom. The layer of mud gave quantitative evidence of the extent to which the lake had been filled in a short time.

A second phase of rapid erosion and source of sedimentation was found along the low cut banks north of the entrance to Muddy Bay (Fig. 7). Here the waves were undercutting the unprotected earthen banks and depositing the clay and topsoil on a low soft fore shore which extended to the permanent silt of the quiet-littoral bottom.

It appears that major undercutting of the banks along this stretch of shore occurs only at peak water levels. Lowering of the water level a foot or so would probably allow the wave action to clean the fore shore of the top layer of clay and re-establish a more typical firm sandy erosion-littoral zone. Nevertheless, each time the water comes to a peak level, many tons of material from these unprotected banks are swept into the lake and thus speed the filling process.

There was yet a third example of aggravated erosion of a perennial type that is well known in Iowa and elsewhere as being especially destructive to aquatic recreational areas. An area of several acres in an arm of Muddy Bay was fenced from the lake and included in a cattle pasture. The pasture was only a moderate sized one and probably was being used as a feeding lot, for a herd of from 30 to 50 cattle was present on August 11, 1954 (Fig. 8). The field and the marshy borders of the arm of Muddy Bay were heavily trampled and the waters were muddy and filthy.

During the summer and fall of 1955, the lake level dropped about 30 inches. The water in Muddy Bay receded so that it did not reach the cattle lot. The farmer extended his electric fence system so that a passageway was opened to the south shore of the entrance to Muddy Bay. There the pollution and trampling was much nearer to the main body of the lake.

Fig. 5. Sheet erosion in the oats field around the inlet into the northern end of North Twin Lake. (May 15, 1954)

FISH FOOD ORGANISMS OF THE EROSION-LITTORAL ZONE

During the summers of 1953 and 1954 bottom fauna were examined from 128 one-quarter square foot Ekman dredge samples from the erosion-littoral zone. The shore samples were taken at depths of from one to two and one-half feet and from 8 to 20 feet from the water's edge. Bottom types were sand of varying degrees of coarseness with some amounts of silt and debris on occasion.

The shore samples were taken to obtain specimens for qualitative comparison with the bottom fauna of the quiet-littoral zone and to determine the habitat location of bottom fauna organisms which were found in fish stomachs.

No estimates were made of the number of 36 square inch Ekman samples needed to obtain a stated degree of accuracy. Tebo (1955) sampled the erosion-littoral zone at comparable depths at Lizard Lake about 15 miles from North Twin Lake with a device which sampled an area of 38.48 square inches. He calculated that 54 such samples were needed to keep the standard error within 10 per cent of the mean.

Fig. 6. Loose earth from excessive erosion from the field shown in Fig. 5 accumulated at the mouth of the culvert leading into North Twin Lake. (May 15, 1954)

The fauna of the erosion-littoral zone of North Twin Lake could be well characterized by the two species which are by far the most prominent. These are nymphs of Caenis sp. (Ephemeroptera) and larvae of Oecetis inconspicua (Walker) (Trichoptera). Caenis sp. occurred in nearly every shore sample, with as many as 17 being taken in one one-quarter square foot haul. Oecetis inconspicua is ideally adapted to the erosion-littoral zone of North Twin Lake, as it makes its case of sand grains cemented together, weighted with a small pebble or two on each side.

A group of exceedingly small Chironomid larvae were present in numbers in the shore zone also. These Chironomidae were one to two millimeters in length, some were green, some whitish or red. They appeared to be different species from Chironomid larvae of the quiet-littoral zone.

Rare organisms of the shore zone included a few Ceratopogonidae, one or two Chaoborus punctipennis, other unidentified diptera larvae and pupae, and a few beetle larvae. Oligochaeta were found in some areas where debris or silt had collected.

Fig. 7. North Twin Lake. Excessive beach erosion and bank sloughing along the western side north of Muddy Bay. (August 11, 1954)

DISCUSSION

The true importance of the erosion-littoral zone in the ecology of the fishes of North Twin Lake would be exceedingly difficult to measure. However, it is believed that the erosion-littoral zone is of very much greater value to the sport fishery than perhaps a quantitative or comparative estimate of the weights of the bottom fauna or fish food produced there might indicate.

The shallows of the erosion-littoral zone provide the essential spawning areas for many species of fish. The young of most species in North Twin Lake are also reared in the shore areas. The erosion-littoral areas are utilized as feeding grounds for intermediate sized yellow perch and yellow bass. Kutkuhn (1954) found the stomach contents of yellow

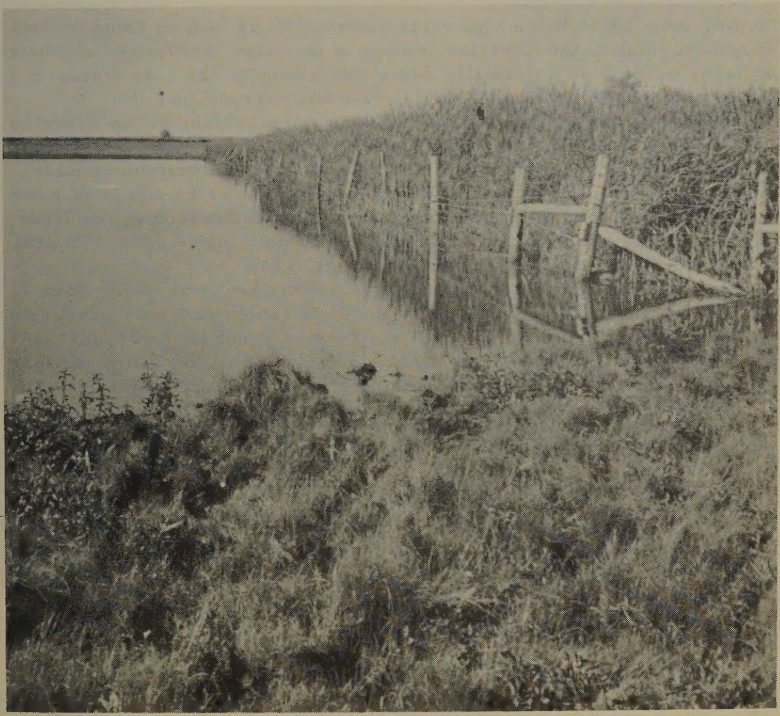

Fig. 8. North Twin Lake. Arm of Muddy Bay fenced from the lake and heavily trampled by cattle. A stand of Typha sp. thrives on the other side of the fence.

perch and yellow bass to include Caenis sp, and Oecetis sp. from the shore zone. Larger individuals of these species and species of game fish such as walleye visit the shore zone to capture young fishes and forage fishes of all species.

A distinction should be made between the stabilized erosion-littoral area which has a clean sandy substrate and the unstable fresh deposits in the erosion-littoral area such as were found at the foot of the cut banks north of Muddy Bay (Fig. 7). Freshly deposited material from eroded soil is almost totally unproductive of fish food. In this respect, the temporary mud flats within the erosion-littoral area should not be confused with the productive stabilized quiet-littoral mud bottom from the same source material.

In summation, it would seem that the greatest factor involving the erosion-littoral zone lying within the scope and power of the fisheries manager would be the stabilization or protection of the zone against undue erosion. Protection against beach erosion and protection of the watershed against sheet erosion would preserve the extent of the sandy

littoral zone which has a high value esthetically as well as being of fundamental value in the fisheries ecology of the lake. Prevention of undue erosion would also materially delay the filling of the lake basin and protect the public investment in this valuable recreational site.

The precipitating cause of the entire problem leading to the dredging of North Twin Lake was obviously the filling of the lake basin. The utmost effort to prevent further siltation would merit serious consideration. The Iowa Twenty-five Year Plan, published by the Iowa State Conservation Department, recommends fencing cattle from public lakes, beach erosion control, and controlling of field erosion in a "Typical Lake Improvement Plan" (Crane and Olcott, 1933).

Most of the immediate watershed surrounding North Twin Lake is privately owned. The fields and pasture, however they may adversely affect the public waters of North Twin Lake, are not subject to management by the state. It would seem that key strips of beach, or sloughs leading into North Twin Lake should be considered for purchase so that permanent erosion control measures could be initiated.

It is perhaps significant that most of the private cottage owners along the eastern shore of the lake have riprapped or built concrete retaining walls along their lake frontage. The erosion-littoral zone along this stretch of beach is wide and clean and quite attractive.

The location of the dredged area seems to have an effect on the width of the erosion-littoral zone. It appears that silt in the south end of the lake near the dredge-cut may settle eventually into the dredged area and be removed. The silt in the north end has no such place to go and repeatedly is stirred up by storms only to settle near shore again at times of calm.

It might seem advisable to dredge a long narrow cut through the long axis of such a lake as North Twin, thereby increasing the width of the clean sandy erosion-littoral zone through the entire length of the lake. Attention should be paid to promontories such as the point and bar near the south entrance to Muddy Bay and the point opposite the state park. The bars out from such points probably should be left undredged; they are swept clean by currents and both areas in North Twin Lake are favorite fishing spots.

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